RESPONSE TO SELECTION AND GENETIC CONTROL OF *IN VITRO* REGENERATION IN ALFALFA (*Medicago sativa* L.)

By

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In memory of my father Erasmo whose life was a hymn of love to his family and to his work .

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Abstract of Dissertation Presented to the Graduate School
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Alfalfa was one of the first major plant species to be successfully regenerated from tissue culture. Somatic embryogenesis has been reported to be heritable and with dominant alleles controlling the trait. Efficient plant regeneration is a prerequisite for use of recombinant DNA technology in alfalfa. The objectives of this study were to develop a nondormant alfalfa population with a high frequency of regeneration and to evaluate the genetic control of this trait both quantitatively and qualitatively. Recurrent phenotypic selection was carried out in two cycles of selection of 'Florida 77', a nondormant alfalfa cultivar. Cotyledon pieces were used to initiate cultures in a three-step protocol: callus growth, embryo induction and embryo development. The selection criteria in cycle 0 was a rating scale from 1 to 3 for the following traits: callus growth, organization (differentiation), and development of structures. In cycles 1 and 2, the number of somatic

embryos/genotype and the number of regenerators were collected. Seedlings from which cotyledon tissue had been removed were grown in the greenhouse for intercrossing between cycles of selection to avoid any aberrant types from regenerated plants. Intercrosses among selections were facilitated by either handcrossing or pollination by leafcutter bees. At the end of the cycle two of selection, plants from all cycles were compared to evaluate the increase in the frequency of regeneration. Quantitative analysis were conducted on data from cycle 1 and cycle 2. Qualitative inheritance was evaluated from crosses among one regenerating genotype and two nonregenerators. Results showed that *in vitro* regeneration capacity had substantially increased after two cycles of selection. Cycle 2 had over 4,800% the number of embryos/genotype compared to cycle 0, and an increase of over 2,000% for the number of regenerators. Combining ability analysis indicated differences among crosses for number of somatic embryos/genotype in cycle 1 but only the GCA effect was significant. Analysis of the half-sib families from cycle 2 for number of somatic embryos/genotype revealed a narrow-sense heritability (h_N^2)=0.49 and a realized heritability (h^2)=0.48. Chi-square analysis of the F_1 generations indicated that a two complementary loci model with additive and dominance effects determined regeneration.

CHAPTER 1
INTRODUCTION

Alfalfa is one of the most valuable forage crops in the world and has been cultivated by humans since ancient times . It represents an important source of proteins, carotene, and minerals, and contains a rich supply of vitamins (Bolton, 1962). The forage is utilized as hay or silage in dairy and beef cattle feed or is dehydrated and used in rations for poultry and other animals.

Cultivated alfalfa (*Medicago sativa* L.) is a self-incompatible autotetraploid species (2n=4x=32), with a perfect flower , and is usually pollinated by bees. Alfalfa improvement has been achieved by traditional breeding, with both interpopulational and intrapopulational selections (Rumbaugh et al., 1988).

Several breeding methods have been used in both intra- and interpopulational procedures. In the interpopulational improvement procedure , plants from one population are crossed with plants from other populations. Reciprocal recurrent selection and topcross selection have been suggested to be more indicated when nonadditive gene action is important (Rowe & Hill,Jr., 1981). Synthetic varieties are the best example of this breeding procedure.

Intrapopulational breeding procedures have been more used to increase pest resistance. Intercrossing resistant plants and reselecting within the resulting populations,

has increased the frequency of desirable alleles to more optimal levels. Individual selection, family selection, and recurrent selection are among the most used methods in this procedure (Rumbaugh et al., 1988). Besides progress in pest resistance, other traits have been improved as a result of breeding efforts, such as forage yield (quantitative and qualitatively), adaptation to specific environments (ex.: salt tolerance), and tolerance to frequent harvests (Hill, Jr., 1987).

A major problem faced by alfalfa breeders is inbreeding depression. Selfing a population for only two or three generations leads to near extinction of the population through loss of vigor and increased sterility (Rumbaugh et al., 1988). Because of the awareness of inbreeding depression and a goal of environmental stability, most synthetic cultivars released in the last 10-15 years have had at least 40 parents (Hill, Jr., 1987). At present, it is common to have 100 or more parents in a cultivar.

In order to overcome problems faced by traditional breeding, alfalfa has been employed in genetic transformation studies. Some successful results have already been reported, like the inclusion of genes coding for disease and herbicide resistance, genes for important proteins, and genes for selectable markers (Samac, 1995).

One key requirement in genetic transformation is a high frequency of *in vitro* regeneration (Bingham et al., 1988). Alfalfa was one of the first major crop species to be regenerated from tissue culture. The efficiency of regeneration has been improved by breeding and by optimization of *in vitro* cultural conditions.

In vitro regeneration in alfalfa occurs mainly via somatic embryogenesis and has been found to be a dominant genetic trait. Two-loci models have been used to explain the

observed segregation ratios between regenerators and nonregenerators (Brown et al., 1995).

Most of the plant culture regeneration studies in alfalfa have involved populations that exhibit some degree of fall dormancy. This is a very complex trait whose expression results from the combined effects of short days and cool temperatures (McKenzie et al., 1988). Nondormant alfalfa, which represents the type of cultivars grown in the deep South of the United States, has received little attention in tissue culture research.

The objectives of this study were to develop a nondormant alfalfa population with high *in vitro* regeneration capacity and to evaluate the genetic control of this trait both quantitatively and qualitatively.

CHAPTER 2
REVIEW OF LITERATURE

Genetics and Breeding in Alfalfa

Alfalfa (*Medicago sativa* L.) originated near Vavilov's "Near Eastern Center" in Asia Minor, Transcaucasia, Iran, and Turkmenistan (Bolton et al, 1972) while *M.sativa* ssp *falcata* originated in the "Central Asiatic Center" in Siberia (Fairey et al., 1996). Despite their different geographic origins, these taxa cross readily and varying degrees of introgression are found in alfalfa cultivars presently grown in North America. Much of the genetic diversity of these alfalfa cultivars is apparently accounted for by nine germplasm sources: *M. sativa* ssp *falcata*, Ladak, *M. sativa* ssp *varia*, Turkistan, Flemish, Chilean, Peruvian, Indian, and African, in descending order of winter hardness (Barnes et al., 1977). The proportion of each of these nine germplasm sources in alfalfa cultivars developed and/or registered for sale in the United States has been recorded most of the time since 1985 (Fairey et al., 1996).

Alfalfa is a perennial self-incompatible species with perfect flowers, naturally cross-pollinated by bees, tolerates comparatively little inbreeding, and can be vegetatively propagated by stem cuttings (Rumbaugh et al.,1988). Alfalfa has a chromosome complement of X=8 with both diploid and tetraploid forms. Cultivated alfalfa is considered

an autotetraploid species since it has 32 chromosomes and exhibits tetrasomic inheritance (Stanford, 1951).

An important consequence of autotetraploid inheritance is that parental inbreeding is partially transmitted to offspring through the diploid gamete. For this reason, genetically broad-based synthetics are very popular in alfalfa breeding, and the proportion of synthetics with more than 40 parents has increased in recent years (Hill Jr., 1987).

According to Hill Jr. (1987), many of the current breeding procedures used in alfalfa were derived from methods used in maize, with modifications to accommodate autotetraploid inheritance and the perfect flower structure of alfalfa. The most common breeding systems are:

1. Recurrent phenotypic selection: this method has proved to be very effective for traits with moderate to high heritabilities and has been used in breeding for resistance to disease and insect pests. In this method, a large number of plants is evaluated for the trait(s) in question, with the selection of the most desirable individuals. These selected individuals are then intermated and the process of selecting and intermating is repeated until the desired level of performance is reached;

2. Backcross breeding: in alfalfa, the recurrent parent, in all cases, and the donor parent, most of the times, are broad-based populations rather than inbred lines in order to avoid inbreeding depression. In this system, donor and recurrent parents are identified, followed by crosses of a large number of plants from each population. The progenies are then evaluated and the best individuals selected. These selections are crossed back to the recurrent parent. The sequence of crossing back a large number of selections to the recurrent parent is repeated until the desired level of the trait(s) is reached;

3. Progeny test selection: the most common in alfalfa is based on half-sib progeny. It can be either an open-pollinated or a polycross progeny test. In either system, a group of parents is identified or selected followed by their intermating. Pollen source is unrestricted in the open-pollinated system, while it is restricted to only selected individuals in the polycross system. The resultant progenies are evaluated and parents of the best progenies are selected.

4. Family selection: it is an extension of the progeny test selection, carried out in a similar fashion except that all individuals or a sample of individuals from the best families are selected.

Intrapopulational selection has been utilized in alfalfa to improve an array of characteristics, most notably in increasing pest resistance (Rumbaugh et al.,1988). Usually, genes for resistance to a specific pest occur at a low frequency in a population. Consequently, breeders have to screen a large number of plants to identify those with resistance. By intercrossing resistant plants and reselecting within the resulting populations, the frequency of genes for resistance has been increased to more desirable levels. The unit of selection may be individual plants or families of plants, depending on the trait of interest.

Fall Dormancy in Alfalfa

Another important aspect of alfalfa is its fall dormancy or winter hardiness. A fall dormancy rating, ranging from 1=dormant to 9=nondormant, was derived from the plant height of regrowth in October after harvest in early September, based on 20 years of data on alfalfa cultivars grown in the United States at Rosemount, Minnesota (Barnes et al.,1978). There was a high correlation between the fall dormancy score and the different proportions of the nine sources of germplasm of each cultivar.

More recently, using restriction fragment length polymorphism among 108 individuals representing the nine original sources of germplasm, Kidwell et al.(1994) observed high levels of genetic diversity among individuals within accessions. When compared with the diversity between accessions, most of the genotypes were not very

distinct genetically. The authors then, concluded that only three distinct germplasm classes can be recognized, namely *M. sativa* ssp *falcata*, Peruvian, and the remaining seven (*M. sativa* ssp *varia*, Ladak, Turkistan, Flemish, Chilean, Indian, and African).

Considering the original nine classes and using information on both the supposed germplasm composition and fall dormancy ratings that have been assigned to alfalfa in North America by breeders, Fairey et al.(1996) did not recognize nine distinct classes of germplasm either, based on partial regression coefficients of fall dormancy. The fall dormancy for North American alfalfa cultivars fall essentially into two classes, namely a dormant category, comprising cultivars containing a large contribution of Falcata and Ladak, and a nondormant category, in which Indian and African germplasm predominates. *M. sativa* ssp *falcata* does appear to be a distinct entity. Ladak is known to be derived from *M. sativa* ssp *falcata*, while *M. sativa* ssp *varia* is a hybrid taxon not well defined yet. The Turkistan, Flemish, Chilean, and Peruvian groups are characterized largely by their route and time of arrival in North America. The Indian and African alfalfa would be derivatives of adaptation of *M. sativa* in warmer latitudes.

Therefore, the classification of alfalfa according to nine dormancy categories has been questioned and the genetic background of different cultivars could, in fact, be similar.

In Vitro Regeneration in Alfalfa

Plant somatic cells that exhibit totipotency, which is the capacity of a cell to generate a whole organism, need to have the gene expression pattern "reprogrammed",

abolishing the previous differentiation cell functions and dedifferentiating. This occurs during the hormone-induced cell divisions, with synthesis of new mRNA molecules during cell division after exposing the somatic cells or tissues to growth regulator (PGR) treatment under *in vitro* conditions. Also, the initiation of the embryogenic pathway is restricted only to certain responsive cells with defined structure and characteristic patterns of cell division. Depending on the interaction between cells and PGR treatment, the subsequent cell divisions can result in either unorganized callus growth or polarized growth with well-coordinated pattern formation resulting in embryo development (Dudits et al. 1995).

In vitro regeneration in alfalfa occurs mainly via somatic embryogenesis (Novak and Konecna, 1982; Atanassov and Brown, 1984; Dos Santos et al., 1983; Li and Demarly, 1995). Somatic embryogenesis is the formation of embryos from somatic cells in a process resembling zygotic embryogenesis. Studies on leaf-derived callus, cotyledon, hypocotyl, root and petiole explants of alfalfa (dos Santos et al.,1983; Wenzel and Brown,1991) show that embryogenic tissue and embryos arise from the tissue surface, and appear to be single cell in origin.

One way of overcoming the limitations faced by traditional alfalfa breeding is to develop engineered transgenic plants carrying desirable traits. Some successful results have already been reported. Novel genes have been introduced into alfalfa (Webb,1986), mainly genes for disease resistance (Hill et al.,1991), genes coding for selectable markers (Deak et al.,1986; Shain et al.,1986; Spano et al.,1987; Chabaud et al.,1988; Du et al.,1994; Desgagnes et al.,1995; Ninkovic et al.,1995), genes for economically important proteins

(Austin et al.,1995), genes for herbicide resistance (D'Halluin et al.,1990), genes for increasing the nodulation by *Rhizobium* (Chabaud et al.,1996).

One of the prerequisites for a successful genetic transformation in plants is a high regeneration capacity of the species or cultivar since the ability of regenerating transgenic plants seems to be highly correlated with high capacity for somatic embryo formation of the genotype (Du et al.,1994; Ninkovic et al.,1995).

Another potential use of somatic embryos is in producing artificial seeds, which consists of somatic embryos enclosed in a protective coating. In alfalfa, the conversion frequencies, i.e., the percent somatic embryos that form complete plants, were 60 to 90% *in vitro* (Redenbaugh, 1990). Due to the difficulty in producing high-quality, vigorous somatic embryos, it is suggested that the application of this artificial seed technology among forage crops is feasible only in alfalfa (Mckersie and Brown, 1996).

So, *in vitro* regeneration in alfalfa occurs mainly via somatic embryogenesis, which has facilitated genetic transformation studies and allowed the production of artificial seeds.

Factors Affecting Somatic Embryogenesis in Alfalfa

The success of embryo induction and plant regeneration are influenced by genotype (Bingham et al.,1975; Walker et al.,1978; Kao and Michayluk, 1980, and 1981; Atanassov and Brown, 1984; Mitten et al.,1984; Brown and Atanassov, 1985; Meijer and Brown, 1985; Chen et al.,1987; Hill et al.,1991), medium protocol (Walker et al.,1978; Walker et al.,1979; Walker and Sato, 1981; Atanassov and Brown,1984; Brown and Atanassov,

1985), and explant source (Novac and Konecna,1982). As in other crops, the interaction among these factors also contributes to the final result (Lazar et al.,1984; Mathias and Simpson, 1986; Bregitzer,1992).

The significant genotype effects suggest that genetic factors are fundamental in the determination of *in vitro* tissue culture response level. Therefore, the first step in *in vitro* culture for crop improvement is to identify plant genotypes that have the capacity for *in vitro* regeneration.

Alfalfa was one of the first major crop plants to be regenerated from tissue culture, with anthers, ovaries, internodes, and seedling hypocotyls used as explant tissue (Saunders and Bingham, 1972). Most of the regenerated plants had the somatic chromosome number of the donor plant even when they were regenerated from anther-derived callus. During proliferation, histological examination of anther-derived callus suggested that most of them were derived from somatic cells in the interlocular connective tissue of the anthers.

Since then, a diverse variety of tissues have been used as explant and regenerated plants, including leaf, petiole, sepal, petal, cotyledon, root, and immature embryos, indicating that there is flexibility in the type of tissue that can be cultured for alfalfa regeneration (Bingham et al. 1988). Recent studies in diploid alfalfa have clearly indicated the influence of the age and physiological state of the explant on regeneration performance (Li and Demarly, 1995, and 1996).

Under the same medium treatments, the response ranged from 0 to 100% of the plants forming embryos, using cotyledon-derived callus and cell suspension cultures of a number of commercial cultivars. But, only two cultivars (Answer and Regen-S) produced

embryos from which plants could be regenerated (Atanassov and Brown, 1984). Using leaf mesophyll protoplasts and cell suspensions derived from shoot tips of an unidentified cultivar, Kao and Michayluck (1981) reported genotypic variation for embryogenesis. Mitten et al.(1984) and Phillips (1983) have also reported results that support the observations on the genotypic variation in regeneration response.

Saunders and Bingham (1972) used clones from the cultivar 'Saranac' and 'Vernal' seedlings in Blaydes's medium supplemented with various combinations and concentrations of kinetin (K), α-naphthalene acetic acid (NAA), and 2,4-dichlorophenoxyacetic acid (2,4-D). They were the first to report on regeneration of alfalfa plants from tissue culture. Immature anthers, immature ovaries, cotyledon, internode sections, and seedlings hypocotyl were used as explants. Immature anthers and ovaries were considered the best tissues for callus formation. Blaydes'medium with 2.0mg L^{-1} each of K, NAA, and 2,4-D gave the best results for callus formation from anthers. Immature ovaries callused well in this combination and also in Blaydes'medium with 0.5mg L^{-1} K and 0.5mg L^{-1} NAA, and with 4.0mg L^{-1} K and 2.0mg L^{-1} NAA. For bud differentiation, Blaydes' medium containing 100mg L^{-1} inositol and 2g L^{-1} yeast extract gave the best results, indicating some genetic specificity for medium. Most of the regenerated plants were tetraploid like the donor plants, but octaploids were also observed. Some off-type plants (dwarf, chlorophyll-deficient and albinos) were also observed. Callus morphology varied among genotypes, indicating that different genotypes may have unique medium requirements for callus development.

Phillips (1983) screened over 300 lines of alfalfas adapted to the southwestern U.S. (nondormant types) for regeneration and found that about 11% of the lines responded positively. No significant difference for regenerator frequency among populations was detected. The optimal regeneration scheme involved callus induction on Schenck and Hildebrandt (1972) medium containing 0.06mg L^{-1} picloran (4-amino-3,5,6-trichloropicolinic acid) and 0.1mg L^{-1} 6-benzylaminopurine(BA) (Phillips and Collins, 1979) and transfer to medium containing 0.01mg L^{-1} 2,4-D(dichlorophenoxyacetic acid) and 2.0mg L^{-1} adenine (Phillips and Collins, 1980) for 1 or 2 culture periods to induce somatic embryogenesis, following the regeneration scheme previously developed for red clover (*Trifolium pratense* L.).

Atanassov and Brown (1984) compared the response of the cultivars Regen-S, Answer, and Citation to *in vitro* regeneration, using leaf hypocotyl and cotyledon explants to derive cell suspensions and callus cultures. Cell division and colony formation were carried out in Kao's medium, whereas a high auxin/low cytokinin treatment on Schenck and Hildebrandt's (1972) followed by culture on growth regulator-free Linsmaier and Skoog's medium resulted in somatic embryo formation. Of the three varieties tested, only the latter two produced embryos from which plants could be regenerated.

Meijer and Brown (1985) screened 19 accessions of diploid *M.sativa* L. germplasm for somatic embryogenesis, using hypocotyl pieces as explants. Two medium protocols were used in a three-step sequence with exposure of the callus cultures to a high 2,4-D concentration (1.0mg L^{-1}) and a two-step sequence without exposure to a high 2,4-D concentration. Great variation among genotypes was observed for embryo production;

however, it was not possible to correlate high regeneration frequencies with a particular germplasm source.

Brown and Atanassov (1985) screened 76 alfalfa cultivars of varying genetic backgrounds for regeneration capacity. A three-step media protocol was used to survey the response of the cotyledons and hypocotyl of each genotype as explant sources. The best regeneration response was observed in creeping-rooted cultivars, especially Rangelander, with 80% of the explants forming embryos. Rangelander contains a large genetic contribution from M. *falcata* and Ladak. However, no consistent correlation between germplasm source and callus production was detected.

Chen et al. (1987) reported quantitative analyses of somatic embryogenesis and plant regeneration in genotypes of three alfalfa cultivars which were all cultured with three medium protocols. 'Heinrichs', 'Rambler' and 'Rangelander' were tested using two types of explant source: cotyledon and leaf pieces in three different media. Somatic embryos were counted and data was expressed as (1) the total number of embryos in each genotype in each medium and (2) the percentage of embryogenic genotypes.. They observed that somatic embryogenesis exhibited a large genotype-medium interaction, but no significant differences between cotyledon and leaf explants were observed with respect to percent of embryogenic genotypes. They also observed that only the embryos which were capable of developing trifoliate leaves were able to grow into plantlets. Between 27 and 71% of the embryogenic genotypes formed trifoliate leaves. In consequence, there was a very low correlation between the number of embryos formed by a genotype and the percent of embryos capable of plant regeneration.

Working on *Medicago suffruticosa* Ramond which is a diploid self-pollinated species, Li and Demarly (1996) observed 18% of regeneration from callus and a histological study showed that this regeneration was through somatic embryogenesis. Pieces of young leaves were used as explant source . The media protocol utilized was: 3-4 weeks in liquid Kao's cell culture medium, containing 4.9uM benzyladenine(BA) and 4.5uM 2,4- D , followed by 4-days treatment in the same medium with 44.4uM BA. The third medium was a free-regulator agar-solidified L1 medium, which is a modified SH medium (Schenk and Hildebrandt, 1972). The authors also observed that calli not exposed to 44.4μM BA for 4 days failed to produce embryos, when transferred to the regulator-free medium. Growth conditions and age of the explant-donor plants were found to have a marked effect on regeneration ability: highest regeneration (18%) was observed from calli derived from young preflowering plants grown with 9h photoperiod and 22^{0}C constant temperature; only 4% of calli derived from equivalent plants grown with a 16h photoperiod and a temperature regime of 17-24°C regenerated into plantlets. Calli derived from fruiting plants grown with a 16h photoperiod did not regenerate plantlets although the callus induction frequency was 99%.

Culture medium has been shown to affect the regeneration response of *Medicago* species. Different salt formulations have been used in successful studies with Murashige and Skoog -based medium formulation being the most often used. Plant growth regulator type and concentration (Saunders and Bingham, 1975; Walker et al., 1978) and nitrogen supply (Walker et al.,1978; Stuart and Strickland, 1984b; Meijer and Brown, 1987b;) are the critical medium components that strongly affect somatic embryogenesis. 2,4-

dichlorophenoxyacetic acid in the 4.5-22.5uM range appears best for embryo induction (Meijer and Brown, 1987a) .

Auxins such as α-naphthalene acetic acid, indole-3-acetic acid and phenylacetic acid, which are not effective at inducing embryogenesis, have been used to propagate cell lines as callus or cell suspensions with low or zero levels of embryo induction. Transfer to a medium containing an effective embryo-inducing auxin (e.g. 2,4-D) for 3-6 days, followed by transfer to a nitrogen-rich medium lacking growth regulators, results in large numbers of somatic embryos (Walker et al, 1979). Cytokinin in combination with an auxin strongly promotes embryo induction (Saunders and Bingham, 1975; Walker et al., 1978; Walker et al., 1979; Meijer and Brown, 1987a; Finstad et al.,1993). Kinetin in the 1-5 uM range has been most often the source of cytokinin used. Induction medium containing kinetin but not auxin did not support callus formation and embryogenesis in alfalfa was not induced. Auxin alone, without kinetin, promoted embryogenesis at a low rate and much later than that observed when 4.7μM kinetin was present (Finstad et al., 1993).

Bingham et al. (1988) have suggested the following guidelines with respect to growth regulator effects in *Medicago*: first, synthetic auxins and cytokinins interact to control morphogenesis; second, a few structural quality differences can be seen among the cytokinins in their effects on morphogenesis; third, the structure of the auxin greatly affects the pattern of morphogenesis; fourth, the response optimum for most growth regulators is broad; fifth, abnormal structures occur when non-optimized levels of growth regulators are employed.

The quantity and quality of medium-supplied nitrogen also appears to be critical for embryo formation in alfalfa (Walker and Sato,1981; Stuart and Strickland, 1984a; Stuart and Strickland, 1984b).The requirement for ammonium ion was confirmed when its addition to the SH regeneration medium restored embryo formation in cells suspension culture. Also, a dosage response of embryogenesis to ammonium ion was observed with maximal response occurring with 12.5 to 25mM NH_4^+ (Walker and Sato, 1981).

Increasing Regeneration Capacity via Somatic Embryogenesis

The *in vitro* regeneration efficiency via somatic embryogenesis in alfalfa is a heritable trait and has been increased by selection and traditional breeding (Bingham *et al.*,1975; Ray and Bingham, 1989; Brown and Atanassov,1985; Bowley *et al.*,1993; Romagnoli *et al.*,1996; Brown,1988).

Bingham et al.(1975) developed a germplasm with a high frequency of regeneration by (phenotypic) recurrent selection . Thirty genotypes derived from Flemish types and thirty 'Vernal' types were screened initially, using Blaydes'medium containing 100mg L^{-1} inositol and 2g L^{-1} yeast extract for budding formation and embryo development. Since regeneration was low and similar for both types, the nondormant cultivars Sonora and 'El Unico' were tested in the same way. Regeneration in Sonora was 17%, similar to Vernal and 'Saranac'. El Unico did not regenerate at all. Then, they tested creeping root cultivars 'Rambler', 'Roamer', and 'Drylander', and 'Rhizoma'. At this time, Rambler and Roamer gave the highest frequency of regeneration, 36% and 35%, respectively. In a further study, they developed Regen-S, using four clones from Saranac and one clone from 'DuPuits'.

These five clones were selected in cycle 0, from the 12% regenerators obtained with Saranac and DuPuits clones. The clones were then randomly intercrossed for the cycle 1 seed, which exhibited an average frequency of regeneration genotypes of 50%. Five vigorous, fertile progenies were selected from each of the five cycle 1 lines, giving 25 clones, which were randomly intercrossed to produce cycle 2 seed. The frequency of regeneration from this second cycle of selection was 67%. Regen-S was then produced by randomly intercrossing three to four vigorous, fertile progenies of 21 lines from the cycle 2 population. Regen-S is similar to Saranac in flower color (blue or purple), which means that it carries at least one dominant P allele for flower color. Pure yellow flowered plants, homozygous recessive for the p allele, were obtained among the Canadian regenerators (Rambler, Roamer, and Drylander). These plants were intercrossed to produce 'Regen-Y'. Hybrids between Regen-S and Regen-Y showed 60% of regeneration frequency, demonstrating that the genetic control of regeneration was similar in both.

Ray and Bingham (1989) obtained a diploid population of alfalfa and studied regeneration ability. Genes controlling regeneration were transferred from tetraploid to diploid plants using haploidy and triploid bridge crosses. The frequencies of alleles controlling regeneration were then increased by two cycles of recurrent selection. The original seed-derived plants, which had served as donors for tissue culture and were capable of regeneration, were recombined in each cycle. This breeding strategy prevented the accumulation of somaclonal variation in the population while increasing the frequency of individuals able to regenerate from tissue culture. The percent of regenerating genotypes increased from 10% in cycle 0 to 33% in cycle 1 , and then, 66% in cycle 2. This study

showed that genes controlling regeneration may be efficiently transferred between ploidy levels and accumulated by recurrent selection and has confirmed the heritability of *in vitro* regeneration.

A backcross procedure was performed to transfer the ability to form somatic embryos from the genotype A70-34, a selection from the cultivar Rangelander, to multiple-pest-resistant alfalfa commercial germplasm. After two cycles of selection (BC_2) the desired level of introgression was only partially fulfilled (Bowley et al.,1993).

Genetic Control of Somatic Embryogenesis

Inheritance studies of tissue culture regeneration are difficult due to the large amount of uncontrolled and environmentally induced variation . It has not always been possible to clearly define what is heritable and what is non-heritable variation. As a consequence, the results reported in the literature are frequently hard to reproduce.

Reisch and Bingham (1980) crossed high (HG2) and low (G11) diploid (2n=2x=16) regenerating genotypes in order to study the qualitative genetic control of (shoot) bud differentiation in alfalfa. Any genotype which regenerated buds after one month of culture in at least one of the three cultures carried out in each genotype, was included in the data used in the calculation of the expected genetic ratios. The parameter used here was the percent of bud differentiation. Since the data did not fit to a single gene model, a two gene model was tested, assuming that the A allele comes from G11 and the B allele from HG2. A pair of complementary dominant alleles (A_B_) at two loci was found necessary for the expression of group A_ type regeneration. The rapid response to selection observed

in specific crosses was consistent with qualitative control of in vitro regeneration. This would make it simpler to transfer genes for regeneration into other genetic backgrounds.

Wan et al. (1988) studied the genetic control of *in vitro* regeneration of tetraploid (2n=4x=32) alfalfa, screening 72 plants from seven cultivars. They used three regenerable plants and one nonregenerable plant from 'Ladak' and one regenerable and two nonregenerable plants from' Lahontan' as parents on various crosses. Ten F_1 populations were derived from crosses between nonregenerable, between regenerable and nonregenerable, and between regenerable plants. Three self populations were derived from selfing of regenerable and nonregenerable plants. A two-step protocol was used in this study: medium 7951 containing 2ml L^{-1} 2,4-D and 0.5mg L^{-1} kinetin was used for callus initiation (1 month). A growth regulator-free medium which was a modified Schenk and Hildebrandt's medium, with the addition of 50uM proline and 30uM alanine was used for regeneration (1 month).Petioles were used as explants and the regeneration efficiency of plants was calculated as percentage of calli regenerating plantlets. The expected segregation ratio of each population was determined from its proposed parental genotype. Segregation of F_1 plants, derived from crosses between two regenerable plants, indicated that callus regenerability was controlled by two dominant complementary alleles at two loci. Based on this hypothesis, the most reasonable genotype was determined for each parent and tested with the segregation ratio in each population. The results suggested that the regeneration of alfalfa via petiole culture was under the control of two complementary alleles and the presence of both dominant alleles at each loci was necessary for a plant to regenerate in a two-step culture system. The data also indicated that gene dosage may have

influenced regeneration efficiency. Since significant reciprocal effects were detected, it was concluded that the interaction between callus induction medium and callus regenerability was affected by cytoplasmic factor(s).

Hernandez-Fernandez and Christie (1989) also studied the inheritance of embryogenic capacity in tetraploid alfalfa. An embryogenic genotype (A70-34), a selection that does not produce callus (MK), and a selection which produces callus but not embryos were used in this study. The plants were self-pollinated and A70-34 was crossed with the other two, including reciprocal crosses. The segregation ratios observed for callus production led to the hypothesis that this trait is controlled by one locus with complete dominance and random chromatid segregation. The data regarding the embryogenic capacity led to the hypothesis that somatic embryogenesis was controlled by two complementary loci, A and B, with additive effects at each locus. The minimum number of alleles for embryogenesis in this model was either one A allele and two B alleles (A _ _ _ BB _ _) or three A alleles and one B allele (AAA _ B _ _ _).

Kielly and Bowley (1992) compared three sources of regeneration (A70-34, C2-4, and RA3) in order to determine if they were controlled by the same genetic system and to confirm that somatic embyogenesis in alfalfa is controlled by two loci. A series of crosses was performed among plants (embryogeneic x nonembryogenic, embryogenic x embryogenic, selfed embryogenic and (embryogenic x nonembryogenic) F_1 crosses backcrossed (BC_1) to the nonembryogenic parent (Excaliber-241). Segregation ratios of most crosses between embryogenic and nonembryogenic genotypes as well as the segregation of the BC_1 progenies was best explained using a two-locus genetic model

(qualitative loci). It was also observed that A70-34, RA3 and C2-4 share the same genetic system.

Crea et al. (1995) studied the constitution of a highly regenerable synthetic population with the genetic background of the cultivar Adriana. Two genotypes (P_1 and P_2) of good regeneration ability were selfed and intercrossed and were crossed with 5 plants randomly taken from the same cv. Adriana, which was determined to be unable to regenerate from callus. Reciprocal crosses were performed. The results indicated that somatic embryogenesis in Adriana was under genetic control. The segregation of progenies from intercrossing or selfing embryogenic genotypes gave a population which segregated following the model of two dominant alleles at two loci proposed by Wan et al. (1988), which contrasts with results by Hernandez-Fernandez and Christie (1989). A maternal effect was also observed: when P_1 or P_2 were the female parent, the percent of embryogenic genotypes increased from 25% to 38%.

Yu and Pauls (1993) studied the segregation of somatic embryogenesis in an F_1 population of 83 plants, derived from a cross between embryogenic A70-34 and non-embryogenic 'Arrow 36' alfalfa plants. The ratio of embryogenic to nonembryogenic genotypes best fitted the two-loci model when the regenerator was used as female parent and the nonregenerator as male parent, with the genotypes being duplex/simplex (AAaaBbbb) and nulliplex/nulliplex (aaaabbbb), respectively.

Therefore, dominant and additive gene action appear to determine *in vitro* regeneration via somatic embryogenesis in alfalfa. Also, a minimum of two complementary loci are reported to be involved in this process.

CHAPTER 3
MATERIALS AND METHODS

Two cycles of phenotypic recurrent selection for *in vitro* regeneration were conducted from October/1994 to December/1996. In order to prevent any deleterious effects from somaclonal variation been introduced, the strategy of recombining seed-derived donors whose explants regenerated in tissue culture (Ray and Bingham, 1989) was adopted in this study. The procedure utilized can be divided in two parts: 1) evaluation of the regeneration capacity and selection of the regenerators in the laboratory, and 2) growth and multiplication of the selections and seed production in the greenhouse.

General Procedure in the Laboratory

'Florida 77', a nondormant alfalfa cultivar was used as the source population in this study. This cultivar was derived from 'Florida 66' with the advantages of being resistant to the spotted alfalfa aphid [*Therioaphis maculata* (Buckton)] and more productive and persistent than Florida 66. The original population from which both Florida 66 and Florida 77 were developed was produced by bulking open-pollinated seed of about 100 varieties and foreign introductions that had been planted in small plots at Gainesville, Fl, in 1950 (Horner and Ruelke, 1980).

A three-step media protocol (callus growth, embryo induction and embryo development) was utilized in the evaluation of the genotypes. Schenk and Hildebrandt basal salts (Schenk and Hildebrandt, 1972) plus growth regulators were utilized in the media (Table 3.1). In the last medium, the high concentration of NH_4^+ (Table 3.1) was achieved with $(NH_4)_2SO_4$. All media pH was adjusted to 5.8 prior to autoclaving for 17 minutes at 20 psi and 121°C, and were solidified with 8g L^{-1} TC agar. Sucrose was used in all media at a rate of 30g L^{-1}. Under aseptic conditions, seeds were sterilized by immersing in H_2SO_4 for 3 minutes, rinsed three times with sterile distilled water, transferred for 3 more minutes to calcium hypochlorite saturated solution, rinsed thoroughly in sterile distilled water, and placed in petri dishes containing seed germination medium (Phillips and Collins, 1979). The dishes were maintained in an incubator for 7-10 days. For callus initiation, from 1/2 to 3/4 of each cotyledon was excised from sterile seedlings and transferred to dishes containing callus initiation medium (SH1) (Table 3.1). The explants were incubated for 3-4 weeks to induce callus formation in a growth chamber. After this culture period, calli were transferred to the induction medium (SH2) (Table 3.1) for 4 days, followed by transfer to the third medium (SH3) (Table 3.1) for embryo development. The production of the seedlings and all cultures were maintained at 26 ± 2^0C with a 16-h day, 85$\mu Em^{-2}s^{-1}$ irradiation supplied by cool-white fluorescent light.

Table 3.1. Growth regulator supplements to the basal media (*) and NH_4^+ used in the three-step protocol with alfalfa.

	Callus initiation (SH1)	Embryo induction (SH2)	Embryo development (SH3)
Growth Regulators			
NAA(0.2mg/l)	25μM	-	-
2,4-D	-	35μM	-
Kinetin(0.125mg/l)	10μM	5μM	-
NH_4^+	-	-	30mM

(*) SH= Schenk & Hildebrandt (1972)

After four weeks on SH3 medium, genotypes were evaluated as follows:

a) Cycle 0: genotypes were evaluated for the presence of somatic embryos. Since few explants produced embryos, the genotypes were rated for callus growth, organization, and presence of structures. Callus growth was classified as little=1, moderate=2, and substantial=3. Organization of the callus was rated as none=1, litttle=2, and substantial = 3. Presence of structures was classified as none=1, green sectors=2, shoots=3s, and roots=3r;

b) Cycle 1: the number of somatic embryos per genotype and the number of genotypes producing one or more somatic embryos (regenerators) were recorded;

c) Cycle 2: the number of somatic embryos per genotype and the number of regenerator genotypes were counted; callus friability and callus color were described; callus size was measured in its diameter, one week after the induction treatment, as follows :

Class	Description
1	no callus
2	callus diameter up to 0.5cm
3	callus diameter between 0.6 and 1.0cm
4	callus diameter between 1.1 and 1.5cm
5	callus diameter between 1.6 and 2.0cm
6	callus diameter between 2.1 and 2.5cm
7	callus diameter between 2.6 and 3.0cm

In all classes, the remaining explant was included in the callus. Callus friability was recorded in three categories at the time of transferring to the development medium as follows: Hard (H) = very difficult to detach cells from the callus surface; Medium (M) = cells could be detached from the callus but an "internal" hard callus was present; Friable (F) = cells very friable, from soft callus, that could be easily removed.

Callus color was recorded before induction and described visually. The number of somatic embryos was recorded for each genotype, at 10x magnification at the end of the third step (development).

General Procedure in the Greenhouse

After removal of cotyledonary tissues for use as explant in the laboratory phase, the seedlings were saved and transferred to Jiffy pellets, acclimatized in the greenhouse, and subsequently transferred to containers with soil for plant growth .

After each laboratory evaluation, seedlings corresponding to cultures that had produced somatic embryos (selections) were transferred to pots for further development. Nonselected seedlings were discarded. Except for cycle 0, explants from all selections produced at least one somatic embryo per genotype.

As only five regenerators were obtained in cycle 0, ten of the best nonregenerators were selected as parents for the intercrosses. These nonregenerator cultures all exhibited some visible structural organization. The inclusion of these ten clones as parents was done in order to reduce any deleterious effect of inbreeding in subsequent generations, which is known to occur in alfalfa.

Parental selections were vegetatively propagated and cuttings were rooted in trays filled with perlite, under high humidity. A rooting compound (Rootone) was applied to enhance rooting. The plants were grown in a greenhouse in pots with soil, at 25-27°C supplied with water and fertilizers as needed.

In cycle 0, selections were handcrossed in all possible combinations, including reciprocal crosses. Flowers were tripped without emasculation of the female parent and pollen transferred from the male plant (Hill ,Jr., 1987). In cycle 1, selections were placed in crossing cages and leafcutter bees[*Megachile rotundata* (F.)] were used to facilitate the pollination.

For both cycles, pods were harvested, kept in the shade, at room temperature, for drying. When dried, seeds were manually extracted using a surface covered with sand paper and a piece of wood covered with rubber. The clean seeds were stored at $\pm 4^{0}$C.

Comparative Study

This study was conducted to measure the genetic gain attained by selection for *in vitro* regeneration. Cycles 0, 1, and 2 were analyzed in a complete randomized design (CRD) with four replications and 60 genotypes per replication per cycle. The genotypes were evaluated for callus size, callus friability, callus color , number of somatic embryos per genotype, and total number of regenerators. Callus friability and callus color were recorded during the transfer from the induction to the development medium.

The number of somatic embryos and the number of regenerators were counted at the end of the last stage. The cycle 2 seedlings were transferred to Jiffy pellets and moved to the greenhouse as they were included in the overall evaluation of cycle 2 plants.

Qualitative Study

In order to study the inheritance of *in vitro* regeneration in this nondormant alfalfa population, the best regenerator (genotype 192) and two nonregenerators (315 and 432) from cycle 0 were intercrossed in all possible combinations, including reciprocals. The resultant seeds were evaluated in the laboratory using 75 genotypes/cross. A genotype was classified as a regenerator if it produced at least one somatic embryo. In a following step, original parents were selfed and the resultant seeds were also evaluated for regeneration capacity.

Statistical Analysis

The response to selection was evaluated for each cycle based on the proportion of regenerating genotypes . In the comparative study, response variables included the number of regenerators /cycle, number of somatic embryos/genotype and callus size and data were exposed to analyses of variance techniques with the means compared using Duncan's procedure. The selection gain, using the data from the comparative study, was calculated for number of somatic embryos/genotype and number of regenerators/cycle, in relation to cycle 0.

Quantitative analyses were performed through a combining ability analyses (Griffing, 1956), using Method 3 in Model 1 (fixed effects) with the data from cycle 1. Components of variance were calculated from cycle 2, in order to evaluate the narrow-sense heritability (h^2_N) (Falconer, 1989). Data from cycles 1 and 2 were used to estimate a realized heritability value (h^2) (Falconer, 1989). Before analyses, data for number of somatic embryos/genotype were transformed using square root of (x+0.5) (Bartlett, 1947), due to the occurrence in many cases of less than 10 embryos/genotype.

Qualitative analysis were performed using the data from the crosses among regenerators and nonregenerators (F_1's and selfed generations). Chi-square tests for goodness of fit were used to analyze the segregation ratios.

The expected segregation ratio of each population was determined from its proposed parental genotype, assuming tetrasomic inheritance.

CHAPTER 4
RESULTS AND DISCUSSION

Response to Selection

Cycle 0

Explants from 534 seedlings (genotypes) were cultured and evaluated for the production of somatic embryos. Due to occurrence of a high level of contamination , 265 genotypes (49.6%) were lost in the first (callus initiation) and second (embryo induction) stages. Of the remaining 269 cultures , only five genotypes (1.9%) produced somatic embryos and were termed regenerators. One hundred and sixty genotypes (59.5%) produced green sectors while 104 (38.7%) developed only callus without any sign of organization or presence of structure (Table 4.1). These last two groups were termed nonregenerators.

Table 4.1. Number of genotypes in different classes of callus growth, callus organization and presence of structures in cycle 0.

	Callus growth [1]			Callus organization[2]			Presence of structures[3]			
Classes	1	2	3	1	2	3	1	2	3s	3r
Number	32	85	152	15	240	14	104	160	5	62

(1) 1= none or little, 2= moderate, 3= substantial ; (2) 1= none, 2= little, 3= substantial; (3) 1= none, 2= green sectors, 3s= somatic embryos, 3r= roots.

Despite the fact that only 5 genotypes produced somatic embryos, 59.5% of the genotypes evaluated exhibited green sectors (Table 4.1). The number of genotypes that formed greater calli (class 3) represented 56.5% of the genotypes evaluated. This means that 'Florida 77' callused well although the regeneration capacity observed was very low in this original population. Roots were observed in 23% of the genotypes evaluated (Table 4.1), most of the times associated with nonregenerators.

Cycle 1

From the original five regenerators from cycle 0, four survived in the greenhouse and were used as regenerator parents. Ten nonregenerators were added to the selections to compose the parental population (14 parents) for cycle 1. The 10 nonregenerators were chosen from the best of the group based on the formation of green sectors. Of 136 crosses attempted, 117 produced sufficient seeds for evaluation in cycle 1. Five genotypes per corss were evaluated for callus growth, callus organization and number of somatic embryos/genotype.

In this cycle, 75 regenerators (12.8%) and 510 nonregenerators (87.18%) were identified. Ten cultures (1.7%) from different genotypes were contaminated in the first stage but, at least one callus from each genotype was saved. Extra dishes (genotypes) from several crosses were also cultured to avoid loss of data.

In this cycle, most of the calli were of small to medium size (classes 1 and 2) (Table 4.2). This was due, in part, to the size of the explant used. They were smaller than the ones in cycle 0.

It was observed that cutting about 3/4 of the cotyledon (done in cycle most of the time), there was a tendency for more rooting. In subsequent cycles, 2/3 or less of the cotyledon was used as explant tissue.

Table 4.2. Number of genotypes in different classes of callus growth and callus organization in cycle 1.

	Callus growth[1]			Callus organization[2]		
Classes	1	2	3	1	2	3
Genotypes	259	236	90	422	140	23

(1) 1= none or little, 2= moderate, 3= substantial
(2) 1= none, 2= little, 3= substantial

Despite the smaller number of calli in class 3, the number of regenerators increased in relation to cycle 0, from 5 to 75, showing the result of the selection applied. Also, it was evident that callus size did not correlate with the formation of somatic embryos, since most of the regenerators were formed from calli from classes 1 and 2 (Table 4.3).

Table 4.3. Number of regenerator genotypes per class of callus growth in cycle 1.

	Callus growth class[1]		
	1	2	3
Number of regenerators	25	34	16

(1) 1= none or little, 2= moderate, 3= substantial

Cycle 2

From the 75 regenerating genotypes identified in cycle 1, 73 genotypes survived in the greenhouse and were used as parents in the next cycle of selection in a polycross mating design. The plants were randomly distributed in cages, in the greenhouse, at approximately the same stage of development (beginning of flowering). Two cages were used, each one containing one clone from each parent. From this polycross, 73 half-sib families produced seeds which were then, evaluated for *in vitro* regeneration. Seven genotypes/half-sib family were tested.

A total of 201 genotypes (39.3%) were identified as regenerators, showing a large increase in the number of regenerators in relation to cycle 0. Only one half-sib family [4x343(3)] failed to produce any visible embryos. Nonregenerating genotypes represented 60.7% of the total. The level of contamination was 1.0%, but it was possible to save or replace the contaminated genotypes.

To better quantify callus development, calli were measured rather than rated visually as done in cycle 0 (Table 4.4).Callus size was distributed among five classes, with predominance of classes 3 and 4, where calli measured 0.6 to 1.0cm, and 1.1 to 1.5cm in diameter, respectively.

Table 4.4. Number of genotypes in different classes of callus diameter (cm) in cycle 2.

Callus class[a]	Number of genotypes	% of each class
1	2	0.39
2	7	1.37
3	247	48.34
4	248	48.53
5	7	1.37

(a) 1= no callus; 2= up to 0.5cm; 3= 0.6-1.0cm; 4=1.1-1.5cm; 5=1.6-2.0cm

The number of somatic embryos/genotype varied among callus size classes, with the majority of genotypes that produced from 0 to 10 somatic embryos/genotype in the classes 3 and 4 (Table 4.5). Among the selected regenerators, 48.2% produced more than 10 somatic embryos/genotype and were concentrated in classes 3 and 4. Therefore, more than 50% of the regenerators selected produced less than 11 somatic embryos/genotype with callus size varying from 0.6 to 1.5cm in diameter. This showed that large callus size was not a prerequisite for somatic embryo formation.

Table 4.5. Number of genotypes according to callus size and number of somatic embryos/genotype in cycle 2.

	Number of somatic embryos/genotype		
Callus size class[a]	0 - 10	11 - 20	>20
1	2	-	-
2	8	-	-
3	198	28	23
4	200	21	25
5	4	2	-

(a) 1= no callus; 2= up to 0.5cm; 3= 0.6-1.0cm; 4= 1.1-1.5cm; 5= 1.6-2.0cm.

The number of somatic embryos /genotype varied from 0 (60.7%) to 81(0.19%), with an average of 1.8 somatic embryos/genotype. When considering only the regenerators (39.3%), the mean number of somatic embryos/genotype was 14.4.

The predominant callus color was clear or light beige (opaque), some with traces of red in their cells. Most calli were friable, and usually the ones that produced somatic embryos were friable (31.7%). Hard calli did not contribute to regeneration capacity, and among the nonregenerators, less than 1% produced this type of calli (Table 4.6).

Table 4.6. Number of genotypes according to their calli friability and regeneration capacity in cycle 2.

Type of genotype	Callus friability		
	Friable	Medium	Hard
Regenerator	162	40	-
Nonregenerator	153	154	2

Comparative Study

This comparative study was performed with the objective of estimating selection progress through two cycles of intrapopulational selection. Callus size was concentrated in the classes 3 and 4 in the three cycles (Table 4.7). Ninety two percent of the genotypes produced callus which measured from 0.6 to 1.5cm in diameter, similar to the result obtained in cycle 2.

Florida77 (cycle 0) showed a greater number of calli in class 5 than the other two cycles, but also had three genotypes that did not form callus (class 1). All genotypes from cycles 1 and 2 callused well. Most of the genotypes exhibited friable callus in all cycles (Table 4. 8). Selection could account for the fact that all genotypes callused in cycles 1 and 2.

Table 4.7. Number of genotypes in different classes of callus diameter (cm) in three cycles of selection.

	Callus size class[a]				
Cycle	1	2	3	4	5
0	3	7	65	71	14
1	0	2	63	93	2
2	0	8	86	65	1

(a) 1= no callus; 2= up to 0.5cm; 3= 0.6-1.0cm; 4= 1.1-1.5cm; 5= 1.6-2.0cm.

Table 4.8. Distribution of the genotypes according to callus diameter and friability in cycles 0, 1, and 2.

	Friability								
	Cycle 0			Cycle 1			Cycle2		
Callus class[a]	F[b]	M	H	F	M	H	F	M	H
1	-	-	-	-	-	-	-	-	-
2	3	3	1	1	1	-	5	3	-
3	33	28	4	43	20	-	62	24	-
4	40	28	3	54	39	-	46	19	-
5	11	3	-	2	-	-	1	-	-

(a) 1= no callus; 2= up to 0.5cm; 3= 0.6-1.0cm; 4=1.1-1.5cm; 5= 1.6-2.0cm.
(b) F= friable; M= medium; H= hard

The number of somatic embryos/genotype varied significantly among cycles (Table 4.9), increasing from 0.07 in cycle 0 to 3.38 in cycle 2 (Table 4.10). This increase corresponded to a selection gain over 4,000% over cycle 0 (Table 4.12).

Table 4.9. Analyses of variance for number of somatic embryos/genotype in two cycles of selection

Source	df	MS	F
Selection cycles	2	66.16	3.00**
Error	477	1.27	

** Significant at 1% level

Table 4.10. Mean number of somatic embryos/genotype and mean number of regenerators/cycle in two cycles of selection (*).

Cycle	Somatic embryos/genotype	Regenerators
0	0.07 b	4.00 c
1	0.48 b	27.00 b
2	3.38 a	81.00 a

(*) Means within a column followed by different letters are significantly different from each other, at 5 % level by Duncan's Multiple Range Test.

The number of regenerators greatly increased with the selection (Table 4.11), going from 1 in cycle 0 to 20.25 in cycle 2 (Table 4.10), corresponding to a selection gain of 2,025% over cycle 0 (Table 4.12). Therefore, the number of somatic embryos/genotype and the number of regenerators/cycle were greatly increased as result of the selection applied.

Table 4.11. Analysis of variance for number of regenerators/cycle in two cycles of selection.

Source	df	MS	F
Selection cycles	2	401.58	4.26**
Error	9	7.53	

** Significant at 1% level

Table 4.12. Selection gain (percent of cycle 0) for number of somatic embryos/genotype and number of regenerators/cycle.

Cycle	Somatic embryos/genotype	Regenerators/cycle
0	100.0	100.0
1	685.7	675.0
2	4,828.6	2,025.0

The proportion of regenerator genotypes (0.9%) in cycle 0 was much less than that reported in initial screening of populations, which has been around 10% of regenerating genotypes (Phillips, 1983; Ray and Bingham, 1989). The number of somatic embryos/genotype increased through the cycles of selection as well the number of regenerators. This reflects the genetic dependence of this trait, since recurrent selection is used in plant breeding to accumulate favorable alleles. The usefulness of this type of plant selection for regeneration capacity in alfalfa was first demonstrated by Bingham et al (1975), when only two cycles of recurrent selection increased the regeneration capacity from 12% to 67%, resulting in the release of the germplasm Regen-S. Later, a comparable success was achieved with diploid alfalfa (Ray and Bingham,1989). In red clover, five

cycles of recurrent selection increased plant regeneration via somatic embryogenesis from callus culture from 4 to 72% (Quesenberry and Smith, 1993).

No relation between callus growth and somatic embryo formation was observed in this study, which is in agreement with most of the literature related to this aspect of tissue culture . Callus growth has not been correlated with plantlet induction or regeneration , and usually, callus was present even when nonregeneration occurred (Reisch and Bingham, 1980; Brown and Atanassov, 1985; Meijer and Brown, 1985; Crea et al., 1995, and Li and Demarly, 1996). But some studies (Walker et al.,1979, and Finstad et al., 1993) have shown a potential relationship between callus growth and regeneration.

Quantitative Study

Data from cycle 1 represented a complete diallel progeny test for the 10 parents, and were subjected to a combining ability analyses (Griffing, 1956). The analyses of variance showed significant difference among crosses for the number of somatic embryos/genotype (Table 4.13).

Table 4.13. Analysis of variance for number of somatic embryos/genotype among 90 single crosses.

Source	df	MS	F
Crosses	89	1.19	1.86*
Error	360	0.64	

* Significant at 5% level

The partition of the crosses into General Combining Ability (GCA) , Specific Combining Ability (SCA), and Reciprocals showed significance only for GCA effects. The SCA and Reciprocal effects were non-significant (Table 4.14). Therefore, genetic effects existed and were due to additive genetic variance for this trait.

Table 4.14. Partition of GCA, SCA, and Reciprocals for number of somatic embryos/genotype.

Source	df	MS	F
Crosses	89		
GCA	9	1.26	1.97*
SCA	35	0.14	0.22
Reciprocals	45	0.09	0.14
Error	360	0.64	

* Significant at 5% level

Since the experimental design was based on parents with the best performance in cycle 0, the range of variation for *in vitro* regeneration capacity was restricted, which explains the small values in the combining ability analyses. With a more representative

sample of plants, greater genetic variation may have been observed. This may have influenced the nonsignificant results for SCA and Reciprocals.

The contribution of the individual genotypes to the F_1s' response was determined by comparing the general combining ability effects. Positive values indicate a contribution towards a greater number of somatic embryos/genotype, while negative values indicate a contribution towards a smaller number of somatic embryos/genotype. (Table 4.15).

Table 4.15. Estimates of general combining ability (GCA) for number of somatic embryos/genotype in alfalfa.

Parent	GCA
4	2.123
192	0.535
343	-0.259
367	2.392
43	-0.592
111	-0.119
167	-1.229
223	-0.621
304	-0.876
466	-1.355

LSD (0.05) = 0.319

The estimates of GCA effects indicated that genotypes 367 and 4 contributed more to greater number of somatic embryos/genotype. Their effects were significantly different

from zero but not different from each other. The genotype 192 also exhibited a significant GCA effect, for the same trait, but at a less extent. This difference, however, was not detected in practice, when the genotypes from cycle 0 were evaluated. At that time, 192 exhibited greater production of somatic embryos than any other and for this reason was chosen as regenerator parent for the qualitative study. Genotype 466 had the highest negative effect on this trait. This suggests that development of genotypes with good number of somatic embryos using methods that exploit additive types of gene action should be effective.

In cycle 2 the analyses of variance showed significant differences among half-sib families only for number of somatic embryos/genotype. Number of regenerators did not differ among the families (Table 4.16).

Table 4.16. Analysis of variance for number of somatic embryos/genotype in cycle 2.

Source	df	MS	F
Half-sib families	72	3.60	1.24*
Error	438	2.89	

* Significant at 10% level

With the values (MS) from this analysis of variance, the variance components were calculated according to Falconer (1989) using the expected mean squares listed in Table 4.17. The error variance is represented by σ_E^2 and the variance among half-sib families by σ_F^2.

Table 4.17. Expected mean squares (EMS) for half-sib families and error variances in cycle 2.

Source	df	E(MS)
Half-sib families	72	$\sigma_E^2 + 7\,\sigma_F^2$
Error	438	σ_E^2

The variance of half-sib families (σ_F^2) was used to calculate the additive genetic variance (σ_A^2). In the case of half-sib families, the genetic variance among half-sib families represents 1/4 of the additive variance (Falconer,1989), and therefore $\sigma_A^2 = 4\,\sigma_F^2$. The phenotypic variance among half-sib family means (σ_P^2) was calculated using the expression $\sigma_P^2 = \sigma_A^2 + \sigma_E^2/7$ (Nguyen and Sleper, 1983). These values were then used to estimate the narrow-sense heritability (h_N^2) for number of somatic embryos/genotype through the expression $h_N^2 = \sigma_A^2/\sigma_P^2$ (Falconer, 1989).

Another way of estimating heritability is calculating the realized heritability (h^2) (Falconer, 1989), which takes into account the amount of selection applied, using the formula $h^2 = [\hat{G} - \bar{x}_{pop}]/[\bar{x}_{sel} - \bar{x}_{pop}]$, where $\hat{G}$ = mean of the new population, $\bar{x}_{pop}$ = mean of the source population, and $\bar{x}_{sel}$ = mean of the parental selections from the source population. In this study, $\bar{x}_{pop}$ came from cycle 1 and $\hat{G}$ was the mean of the cycle 2 population.

The narrow-sense heritability for number of somatic embryos/genotype was 0.49. Since this value "expresses the extent to which phenotypes are determined by the genes transmitted from the parents" (Falconer, 1989, p. 126), this value can be considered high,

what indicates that the trait in question is highly heritable. The estimate of the realized heritability for the same trait was 0.48 and can also be considered high. Realized heritability takes into account the selection applied. It is based on actual gain, being primarily a description of the response obtained with the selection (Falconer,1989).

Both heritability estimates were similar, which indicates that neither method was biased. When estimating narrow-sense heritability by the components of variance, one of the assumptions is that there is additivity of genic effects. This was detected in the combining ability analysis (Table 4.14). The response obtained showed an increase in the number of somatic embryos/genotype and in the number of regenerators (Tables 4.10 and 4.12), confirming that favorable alleles had been incorporated into the genotypes tested.

Quantitative genetic studies of *in vitro* regeneration capacity are scarce. Most of the observed genotypic variation for somatic embryogenesis induction has been reported to be mainly due to additive effects in wheat (Ou et al., 1989), red clover (Keyes et al.,1980), pea (Bencheikh and Gallais, 1996), maize (Tomes and Smith, 1985) , and triticale (Charmet and Bernard, 1984). Additive and dominance effects have been reported in barley (Hou et al., 1994), and melon (Molina and Nuez, 1996) . Estimated heritabilities were high for plant regeneration response in maize (Tomes and Smith, 1985), wheat (Lazar et al.,1984), rice (Miah et al, 1985), red clover (Quesenberry and Smith, 1993), and *Cucumis sativus* L. (Nadolska-Orczyk and Malepszy, 1989).

The main objective of a recurrent selection program is to alter the population mean for a character resulting in improved performance of the crop. In a phenotypic recurrent selection program, it is important to have high heritability of the trait(s) under study so the

progress can be achieved more rapidly independent of the influence of the environment. The results of this quantitative study show that the breeding method adopted was effective, *in vitro* regeneration in nondormant alfalfa was under genetic control, and highly heritable, which agrees with a previous observation (Bingham et al.,1975) in dormant alfalfa.

Qualitative Study

The F_1 families derived from the crosses involving one regenerating genotype (192) and two nonregenerators (315 and 432) were scored for callus growth, callus organization and number of somatic embryos/cross and the selfed families were scored for callus size (diameter), callus friability, number of somatic embryos/genotype, and number of regenerators.

Callus growth showed similar distribution of the genotypes in different crosses, with a majority in classes 1 and 2 (little and moderate growth, respectively). Some difference in this distribution was observed between the progeny from the cross 192 x 315 and the progeny of its reciprocal. Progenies in the former cross showed greater number of calli in class 1 than those in the reciprocal. The same was true, in the opposite direction, in relation to class 3. Chi-square analysis of the distribution of the genotypes in the F_1 families showed significant difference for callus growth between the reciprocal crosses involving 192 and 315 parents. This suggests the occurrence of maternal effect in the cross 192 x 315 and its reciprocal for this trait (Table 4.18). Callus organization was concentrated in class 1 (no organization), but again the cross 315 x 192 yielded more regenerators then its reciprocal (Table 4.19). Chi-square analysis of the distribution of the genotypes in the F_1 families also

showed significant difference between the reciprocals crosses involving 192 and 315 (Table 4.19). Then, this result also suggest the occurrence of maternal effect in the cross 192 x 315 and its reciprocal for callus organization. The results in general were similar to that of cycle 1 (Table 4.2), where the same criteria were used for callus growth and organization. The differences between the reciprocal crosses involving 192 and 315 do suggest the occurrence of some maternal effect, although reciprocity was not detected in the combining ability analysis (Table 4.14).

Table 4.18. Genotype distribution and χ^2 values for callus growth in six F_1 families.

	Callus organization[1]			χ^2	p
Cross	1	2	3		
192 x 315	18	47	7	18.97	<0.001
315 x 192	4	46	25		
192 x 432	19	36	20	3.41	0.30-0.20
432 x 192	15	47	13		
315 x 432	25	35	15	0.69	0.90-0.70
432 x 315	25	38	11		

(1) 1= none; 2= little; 3= substantial.

Table 4.19. Genotype distribution and χ^2 values for callus organization in six F_1 families.

	Callus organization[1]			χ^2	p
Cross	1	2	3		
192 x 315	41	24	7	9.58	0.01 - 0.001
315 x 192	40	14	21		
192 x 432	59	14	2	4.10	0.20 - 0.10
432 x 192	53	22	-		
315 x 432	66	8	1	3.28	0.20 - 0.10
432 x 315	68	3	2		

(1) 1= none; 2= little; 3= substantial.

The S_1 progenies callused well on the medium used in this study. They varied in callus size, with most of the calli in groups 3 and 4 (Table 4.20), similar to the distribution observed in cycle 2 (Table 4.4).

Table 4.20. Distribution of 50 progenies in each of three selfed families for callus size and callus friability.

	Callus size (cm)[1]							Friability(%)[2]		
Family	1	2	3	4	5	6	7	F	M	H
192	-	-	39	10	1	-	-	70	28	2
315	-	-	13	33	4	-	-	14	82	4
432	-	-	7	39	4	-	-	18	64	18

(1) 1=no callus, 2= up to 0.5cm, 3=0.6 to 1.0cm, 4=1.1 to 1.5cm, 5=1.6 to 2.0cm, 6=2.1 to 2.5cm, 7=2.6 to 3.0cm.

(2) classes of friability: F= friable, M= medium friability, H= hard.

The selfed progeny from the regenerator (192) had greater number of friable calli while the nonregenerators produced more calli of medium friability (Table 4.19). Also, all the 12 regenerators from the 192 selfed progeny produced friable calli. The only regenerator from selfed progeny of 432 was of medium friability.

Comparing the callus growth among the selfed progenies, it can be noted that 192 produced a greater number of friable calli while 315 and 432 had more calli of medium friability. This difference might be related to their capacity for forming somatic embryos, which was greater in 192, insignificant in 432 and did not exist in 315 (Table 4.20). These results suggest that callus friability is directly related to regeneration capacity. And this is a very important aspect of callus formation, because it influences directly the establishment of suspension cell cultures (Arcioni et al. 1990).

Crosses involving 192 and 315 produced higher number of somatic embryos per genotype than the selfs of 192, but the selfed progenies from 192 was not different from those ones from 315 and 432 (Table 4.21). This could reveal the occurrence of heterosis, with overdominance in that crosses, for this trait. Inbreeding depression could also account for the low number of somatic embryos/genotype in the 192 selfed progeny.

Inbreeding depression has been reported to occur after a few generations of selfing in autotetraploid alfalfa (Busbice and Wilsie,1966). Callus production of autotetraploid alfalfa has also been observed to be reduced probably due to inbreeding depression (Keyes and Bingham, 1979).

In this study, callus size was not observed to be reduced by one generation of selfing when compared to callus size of populations after two cycles of selection (Tables 4.4 and

4.19). But, it is possible that selfing reduced the capacity of regeneration. It was, in fact, noted that in cycle 2, the development of callus from crosses involving 192 was slower than others, in many cases, although this delay was not measured. So, 192 could necessitate longer term or more passages into the induction medium to fully develop its high regeneration frequency. Maybe, for this reason the selfed progeny from 192 showed fewer regenerators, when compared to the crosses involving 192 and 315.

Table 4.21. Means for number of somatic embryos/genotype from F_1's and self progenies.

Cross	Mean number of somatic embryos/genotype(*)
192 x 315	5.40 a
315 x 192	5.09 a
192 x 432	0.08 b
432 x 192	0.61 b
315 x 432	0.03 b
432 x 315	0.15 b
192 x 192	1.04 b
315 x 315	0.00 b
432 x 432	0.42 b

(*) Means within a column followed by common letters were not significantly different at $p \leq 0.05$ by Duncan's Multiple Range Test.

Differences between 315 and 432 were present because 432, when selfed, produced embryos while 315 did not (Table 4.20). In cycle 0, the original regenerator (192) showed substantial callus growth and callus organization and production of somatic embryos, while

315 showed substantial callus growth, presence of green sector, but no somatic embryos. The genotype 432 showed substantial callus growth but no organization and no embryo. Therefore, all three genotypes were similar in terms of callus growth, differing in callus organization and in the production of somatic embryos. The production of somatic embryos in the crosses (192x315) and (315x192) suggests that there is a dominant effect for somatic embryo production. Since the self of 315 did not produce any embryo, this would be the recessive genotype. The same is not true when considering the crosses (192x432) and (432x192). There appears to be some mechanism preventing somatic embryo formation in a larger scale in these crosses. This could be due to modifying genes present in 192 or 432, that are not expressed when combined with 315.

Modifying genes are genes that intensify or diminish the expression of a major gene, altering the expected Mendelian segregation ratios. They were first detected in rats and have been reported to cause great variability on the expression of genes in many crops, with their action affecting traits like plant height, flower and fruit characteristics, and disease resistance (Briggs & Knowles, 1967). More recently, studies have reported the occurrence of modifying genes in maize, soybean, barley (Thomas et al.,1994, Mickelson & Rasmusson, 1994, and Hohls et al., 1996), among others. They can occur in F1's and be detected only after several generations of backcrosses, when their effects have been suppressed (Briggs & Knowles, 1967).

Segregation ratios of crosses between regenerator and nonregenerators, between nonregenerators, and selfed progenies of the regenerator and nonregenerators best fitted a two-loci genetic model with additive and dominance gene effects, according to the Chi-

square analyses (Table 4.21). Genotype 192 segregated according to a genotype of AAaaBbbb when crossed with 315(aaaaBbbb) and with 432 (aaaabbbb) when it was used as female parent. The same genotype, however, failed to fit the segregation ratio of its selfed population.

Table 4.22. Segregation ratios of the F_1 and self populations considering a two-loci with additive gene action in one locus and dominance at the other locus.

Cross R:NR	Observed ratio R:NR	Expected ratio R:NR	χ^2	p	Proposed genotypes
192 x 192	12:38	89:55	30.26	<0.001	AAaaBbbb x AAaaBbbb
315 x 315	0:50	0:1	-	-	aaaaBbbb x aaaaBbbb
432 x 432	1:49	0:1	-	-	aaaabbbb x aaaabbbb
192 x 315	21:51	7:17	0.0	∞	AaaaBbbb x aaaaBbbb
315 x 192	24:51	7:17	0.29	0.70-0.50	aaaaBbbb x AAaaBbbb
192 x 432	10:125	1:11	0.15	0.70	AAaaBbbb x aaaabbbb
432 x 192	19:116	1:11	5.82	0.05-0.01	aaaabbbb x AAaaBbbb
315 x 432	2:73	0:1	-	-	aaaaBbbb x aaaabbbb
432 x 315	1:73	0:1	-	-	aaaabbbb x aaaaBbbb

These results demonstrated that this model was acceptable in all populations but population derived from the cross (432x192) and the 192 selfed progeny. In all other populations the observed segregation ratios corresponded well to the expected ones.

Genotype 192 did have a GCA effect significantly different from zero (Table 4.15). According to the GCA values, the best genotypes for crossing would be 4 and 367. In fact,

these two genotypes did very well in terms of plant development, seed production and regenerable genotypes from cycle 1.

Genotype 4 was a parent in 15 of the selections, and genotype 367 was a parent in 17 of the selections, while genotype 192 was a parent in only 9 selections. But, at the time the parents were chosen to develop the inheritance study, 192 was considered the best in terms of somatic embryo production and plant regeneration.

Kiely & Bowley (1992) have also observed an embryogenic genotype (RA3) which fitted a two-loci model, segregating according to a simplex/simplex genotype of AaaaBbbb, but failed to fit any of the one-or two-loci models tested for the segregation of its self. The reason for this behavior, according to the authors, could be the very narrow germplasm base of RA3, and one generation of selfing could have promoted a strong inbreeding depression in this trait.

In this study, an intrapopulational breeding system was developed, using as source population a nondormant alfalfa cultivar. Although Florida 77 has been developed from about 100 varieties and foreign introductions (Horner & Ruelke, 1980), its specific genetic background cannot be determined. Since recent studies have concluded that the alfalfas cultivated in the United States belong to two or three different genetic groups only, (Kidwell et al.,1994, and Fairey et al.,1996), it is possible that this nondormant cultivar contains fewer genes to promote *in vitro* regeneration.

It has been reported that creeping rooted alfalfas, the most dormant types, are more likely to regenerate well in tissue culture (Bingham et al.,1988). The smaller progress observed in this study when compared to studies with dormant material (Bingham et al.

1975) is in accordance with this hypothesis, i.e., nondormant alfalfa might have less potential for increasing regeneration capacity via traditional breeding.

Regeneration capacity has been reported to be controlled by dominant nuclear genes in dormant alfalfa (Reisch & Bingham, 1980, Wan et al.,1988, Ray & Bingham, 1989, Hernadez-Fernandez & Christie, 1989, Kielly & Brown, 1992, Yu & Pauls, 1993, Crea et al.,1995), *Solanum phureja* (Chen & Veillex, 1991), *Cucumis sativus* L.(Nadolska-Orczyk & Malepszy1989), maize (Rhodes et al., 1986), and tomato (Koorneef et al, 1987). Some results have shown complementarity of the loci in alfalfa (Wan et al, 1988, Hernandez-Fernandez & Christie, 1989).

Although most research results show a dominance mode of inheritance, it has also been hypothesized that other factors can be present influencing *in vitro* regeneration efficiency in alfalfa. In this study the segregation ratios of the crosses between 315 and 432 produced three regenerators when none were expected, according to the proposed model. Also, the selfed from 432 produced one regenerator (Table 4.22). Dosage effects (Wan et al, 1988, Kielly & Bowley, 1992), maternal effects (Crea et al, 1995), and modifying genes (Wan et al, 1988, Hernandez-Fernandez & Christie, 1989) have been considered as possible causes for the deviation of some observed segregation ratios and could have contributed to the formation of somatic embryos in these cases.

Since this was the model that best fitted the data, it is possible that modifying genes were the factor altering the expected ratios of these segregations. A more conclusive answer could be obtained with more advanced generations of selfings or backcrosses involving these genotypes.

CHAPTER 5
SUMMARY AND CONCLUSIONS

With the objectives of developing a nondormant alfalfa population with high *in vitro* regeneration capacity and to evaluate the genetic control of this trait both quantitatively and qualitatively, an intrapopulational selection procedure was developed from October/1994 through December/1996. Two cycles of phenotypic recurrent selection were performed using alfalfa Florida 77 as the source population.

Cotyledon pieces were used to initiate cultures in a three-step media protocol: callus growth, embryo induction, and embryo development. SH basal salts containing NAA at the concentration of 25μM for callus growth, 2,4-D at the concentration of 35μM for embryo development and kinetin at the concentration of 10μM for callus growth and 5μM for embryo induction, were utilized in this study. The last medium, a hormone-free medium, contained NH_4^+ at the concentration of 10mM to enhance regeneration. The genotypes remained 4 weeks in the first medium, 4 days in the second medium, and 4 weeks in the last medium. After this time, they were evaluated for regeneration as follows: a) cycle 0: callus growth, callus organization and presence of structures, in a rating scale of 1 to 3; b) cycle 1: number of somatic embryos/genotype and number of regenerators; c) cycle 2: number of somatic embryos/genotype, number of regenerators, callus size (diameter), callus friability and callus color.

After the evaluation in the laboratory, seedlings corresponding to cultures that had produced somatic embryos (selections) were allowed to grow in a greenhouse and develop plants for future crosses. Ten parents selected in cycle 0 were used to start the selection procedure. They were handcrossed by tripping the flowers, without emasculation of the female parents. In cycle 1, 73 selections were placed in crossing cages and leafcutter bees were used to facilitate the pollination.

Response to selection was evaluated in each cycle for number of regenerating genotypes (cycles 0, 1, and 2) and for number of somatic embryos/genotype (cycle 2) as well as by the genetic gain, estimated in a comparative study of the cycles of selection. The genetic control of *in vitro* regeneration was evaluated from the data collected from the crosses among one regenerator and two nonregenerators and their selfed progenies.

Analysis of the data from individual cycles showed 1.9%, 12.8%, and 39.3% of regenerating genotypes in cycles 0, 1, and 2, respectively. The number of somatic embryos/genotype increased from 0.07 in cycle 0 to 3.8 in cycle 2, corresponding to a selection gain of 4,828.6% over cycle 0. The number of regenerators increased from 1 per 40 individuals to 20.25 per 40 individuals in cycle 2, with a selection gain of 2,025% over cycle 0. The predominant callus size varied from 0.6 to 1.5cm in diameter, and did not show correlation with the number of somatic embryos/genotype. Most of the regenerators were produced from friable calli.

General combining ability was detected for number of somatic embryos/genotype, but no differences were observed for specific combining ability or for reciprocal effects. Narrow-sense heritability for this trait, estimated from the components of variance was 0.49

and the realized heritability was 0.48. The segregation ratios observed best fitted a model of two complementary loci, with additive and dominant gene effects controlling regeneration.

Based on the results obtained in this study, it was possible to conclude that:

1) Florida 77, a nondormant alfalfa cultivar, was amenable to phenotypic recurrent selection for *in vitro* regeneration;

2) Regeneration capacity showed high heritability values, due mainly to additive genetic variance;

3) Regeneration capacity was increased up to 39% after two cycles of selection.

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APPENDIX
DATA

Cycle 0

Genotype	Entry	C.growth	C.organizat.	Structures	Shoots
fl77	4	2	2	3	s
fl77	367	2	2	3	s
fl77	34	3	2	2	
fl77	343	2	2	3	s
fl77	192	3	3	3	s
fl77	226	3	3	2	
fl77	244	2	3	2	
fl77	43	3	3	3	
fl77	234	3	3	3	
fl77	320	3	3	2	
fl77	60	3	3	2	
fl77	223	1	2	2	
fl77	239	3	2	2	
fl77	126	2	3	2	
fl77	111	2	2	2	
fl77	5	1	3	2	
fl77	304	3	2	2	
fl77	167	3	2	2	
fl77	276	3	2	2	
fl77	194	3	2	2	
fl77	466	3	2	2	
fl77	236	3	1	1	
fl77	340	3	1	1	
fl77	315	3	2	1	
fl77	432	3	1	1	
fl77	496	1	1	1	
fl77	372	3	1	1	
fl77	408	3	1	1	
fl77	479	1	1	1	
fl77	225	3	1	1	
fl77	404	3	2	1	
fl77	375	3	1	1	
fl77	161	1	2	1	
fl77	2	3	2	1	
fl77	264	3	2	1	
fl77	362	3	2	2	
fl77	193	2	2	2	
fl77	227	3	2	2	
fl77	68	3	2	2	
fl77	184	3	2	3	
fl77	455	3	2	2	
fl77	323	3	2	2	
fl77	163	3	2	2	
fl77	341	3	2	2	
fl77	353	3	2	2	
fl77	309	3	2	2	
fl77	57	3	2	3	
fl77	168	3	1	1	
fl77	127	3	2	1	
fl77	88	3	2	3	

f177	436	2	2	1
f177	300	3	2	1
f177	301	2	2	2
f177	302	3	2	1
f177	9	2	2	3
f177	8	3	2	3
f177	18	3	2	3
f177	19	2	2	3
f177	39	2	2	1
f177	42	3	2	3
f177	25	3	1	3
f177	252	3	2	2
f177	358	2	2	2
f177	316	3	2	2
f177	66	3	2	2
f177	188	2	2	2
f177	14	2	3	2
f177	338	3	2	2
f177	189	2	2	1
f177	142	1	2	1
f177	1	1	2	3
f177	164	3	2	2
f177	240	3	2	2
f177	3	3	2	3
f177	491	2	2	2
f177	250	3	2	2
f177	261	1	2	2
f177	258	3	2	2
f177	327	1	2	2
f177	292	3	2	2
f177	158	3	2	2
f177	325	3	2	2
f177	324	3	2	2
f177	115	3	2	2
f177	459	2	2	2
f177	452	3	2	2
f177	155	3	2	2
f177	148	2	2	2
f177	350	3	2	1
f177	361	2	2	2
f177	200	3	2	2
f177	305	3	2	2
f177	235	3	2	2
f177	379	3	2	2
f177	271	3	2	2
f177	294	3	2	2
f177	266	3	2	2
f177	130	2	2	2
f177	53	3	2	2
f177	291	3	2	2
f177	431	3	2	2
f177	360	3	2	2
f177	336	3	2	2
f177	480	2	2	2
f177	370	3	2	2
f177	257	2	2	2
f177	275	3	2	2
f177	196	3	2	2
f177	369	3	2	2
f177	56	3	2	2
f177	265	3	2	2
f177	30	3	2	2
f177	453	2	2	2
f177	27	1	2	2
f177	26	2	3	2

f177	295	3	2	2
f177	285	2	3	2
f177	354	3	3	2
f177	159	3	2	2
f177	201	3	2	2
f177	195	3	2	2
f177	185	2	2	2
f177	484	2	2	2
f177	112	2	2	2
f177	197	3	2	1
f177	312	3	3	2
f177	147	2	2	2
f177	251	2	2	2
f177	451	3	2	2
f177	253	3	2	2
f177	307	2	2	2
f177	190	2	2	2
f177	277	3	2	2
f177	284	1	2	2
f177	460	3	2	2
f177	186	1	2	2
f177	22	2	2	2
f177	31	3	2	2
f177	24	2	2	2
f177	475	3	2	2
f177	386	2	2	2
f177	346	2	2	2
f177	501	2	2	2
f177	493	2	2	2
f177	495	2	2	2
f177	427	1	2	2
f177	437	1	2	2
f177	481	2	2	2
f177	487	2	2	2
f177	483	3	2	2
f177	519	2	2	2
f177	534	3	2	2
f177	535	2	2	2
f177	472	1	2	1
f177	527	3	2	2
f177	510	2	2	2
f177	514	3	2	2
f177	398	3	2	2
f177	489	3	2	2
f177	447	2	2	2
f177	388	2	2	2
f177	389	2	2	2
f177	392	3	2	2
f177	428	3	2	2
f177	464	3	1	1
f177	509	1	2	2
f177	463	2	2	2
f177	502	1	2	2
f177	503	1	2	2
f177	517	1	2	2
f177	523	1	2	2
f177	396	2	2	2
f177	400	3	2	2
f177	403	3	2	2
f177	406	3	2	2
f177	507	1	2	2
f177	435	1	2	2
f177	439	3	2	2
f177	521	2	1	1
f177	442	1	2	2

fl77	444	3	2	2
fl77	477	2	2	2
fl77	385	2	2	2
fl77	393	3	2	2
fl77	337	3	2	1
fl77	377	2	2	2
fl77	357	2	2	1
fl77	356	3	2	1
fl77	381	3	2	2
fl77	380	3	2	2
fl77	144	2	2	1
fl77	321	2	2	1
fl77	318	2	2	1
fl77	317	3	2	1
fl77	371	3	2	1
fl77	384	3	2	1
fl77	383	3	2	2
fl77	382	3	2	1
fl77	348	3	2	1
fl77	347	3	2	2
fl77	345	3	2	1
fl77	322	3	2	2
fl77	286	2	2	1
fl77	283	3	2	1
fl77	274	2	2	1
fl77	246	2	2	3
fl77	355	3	2	2
fl77	12	2	2	1
fl77	10	3	2	3
fl77	20	3	2	3
fl77	23	3	2	1
fl77	273	3	2	1
fl77	349	2	2	2
fl77	366	3	2	1
fl77	365	2	2	1
fl77	272	3	2	1
fl77	270	2	2	2
fl77	363	3	2	3
fl77	267	3	2	1
fl77	279	1	1	1
fl77	376	2	2	2
fl77	374	2	2	1
fl77	268	3	2	1
fl77	224	3	2	1
fl77	222	2	2	1
fl77	199	3	2	2
fl77	297	2	2	2
fl77	296	1	2	2
fl77	334	3	2	1
fl77	330	2	2	1
fl77	329	3	2	1
fl77	328	3	2	1
fl77	326	2	2	1
fl77	344	3	2	1
fl77	339	1	2	2
fl77	233	2	2	3
fl77	203	2	2	2
fl77	231	2	2	3
fl77	243	3	2	2
fl77	62	2	2	3
fl77	118	1	2	1
fl77	117	2	2	1
fl77	110	2	2	1
fl77	108	1	2	1
fl77	131	1	2	2

fl77	128	1	2	1
fl77	151	3	2	1
fl77	37	3	2	3
fl77	51	3	2	1
fl77	49	2	2	1
fl77	48	3	2	1
fl77	221	2	2	1
fl77	207	1	1	1
fl77	204	2	2	1
fl77	28	3	2	3
fl77	41	2	2	2
fl77	40	2	2	3
fl77	38	3	2	3
fl77	241	3	2	1
fl77	256	3	2	1
fl77	249	2	2	1
fl77	287	3	2	1
fl77	54	3	2	1
fl77	191	3	2	1
fl77	7	1	2	2
fl77	45	3	2	3
fl77	44	3	2	3
fl77	58	3	2	3
fl77	55	2	2	2

Cycle 1

Cross	Entry	C. growth	C. organizat.	Structures	# SE(transformed)
4x192	1	3	2	3	3.535534
4x192	2	3	3	3	3.535534
4x192	3	1	1	1	.707107
4x192	4	2	2	3	1.870829
4x192	5	3	1	1	.707107
4x367	1	1	1	1	.707107
4x367	2	2	3	2	.707107
4x367	3	2	1	1	.707107
4x367	4	2	2	2	.707107
4x367	5	3	3	3	7.648529
4x304	1	2	1	1	.707107
4x304	2	1	1	1	.707107
4x304	3	2	2	2	.707107
4x304	4	1	2	2	.707107
4x304	5	3	3	3	2.738613
4x343	1	1	1	1	.707107
4x343	2	1	1	1	.707107
4x343	3	1	2	3	1.224745
4x343	4	1	1	1	.707107
4x343	5	2	2	3	2.121320
4x223	1	1	1	1	.707107
4x223	2	1	1	1	.707107
4x223	3	2	1	1	.707107
4x223	4	2	1	1	.707107
4x223	5	2	2	3	2.121320
4x111	1	1	1	1	.707107
4x111	2	1	1	1	.707107
4x111	3	2	3	3	4.183300
4x111	4	2	1	1	.707107
4x111	5	2	2	3	1.581139
192x4	1	1	1	1	.707107
192x4	2	1	1	1	.707107
192x4	3	2	1	2	.707107
192x4	4	3	2	3	2.345208
192x4	5	3	3	3	4.062019
192x223	1	2	1	1	.707107
192x223	2	2	2	2	.707107

192x223	3	2	2	2	.707107
192x223	4	2	2	2	.707107
192x223	5	2	1	1	.707107
192x304	1	2	1	1	.707107
192x304	2	1	2	2	.707107
192x304	3	1	1	1	.707107
192x304	4	2	2	2	.707107
192x304	5	2	1	1	.707107
192x367	1	2	2	3	4.527693
192x367	2	2	2	3	2.121320
192x367	3	2	2	3	3.082207
192x367	4	1	2	3	1.581139
192x367	5	3	2	3	1.581139
223x192	1	1	2	2	.707107
223x192	2	1	1	1	.707107
223x192	3	2	3	3	3.535534
223x192	4	2	2	2	.707107
223x192	5	1	1	1	.707107
223x4	1	2	2	2	.707107
223x4	2	2	2	3	1.870829
223x4	3	2	1	1	.707107
223x4	4	3	2	3	4.527693
223x4	5	1	1	1	.707107
223x367	1	3	2	3	2.549510
223x367	2	2	1	1	.707107
223x367	3	1	1	1	.707107
223x367	4	1	1	1	.707107
223x367	5	1	2	2	.707107
111x4	1	3	2	2	.707107
111x4	2	2	1	1	.707107
111x4	3	2	1	1	.707107
111x4	4	1	1	1	.707107
111x4	5	2	3	3	3.937004
111x192	1	1	1	1	.707107
111x192	2	2	1	1	.707107
111x192	3	2	1	1	.707107
111x192	4	2	2	3	1.581139
111x192	5	2	2	2	.707107
111x367	1	2	2	3	2.915476
111x367	2	2	1	1	.707107
111x367	3	3	2	3	1.224745
111x367	4	2	1	1	.707107
111x367	5	1	1	1	.707107
111x223	1	1	1	1	.707107
111x223	2	1	2	3	1.224745
111x223	3	1	1	1	.707107
111x223	4	2	1	1	.707107
111x223	5	1	1	1	.707107
43x367	1	2	1	1	.707107
43x367	2	2	2	3	1.581139
43x367	3	3	3	3	1.224745
43x367	4	2	1	1	.707107
43x367	5	1	1	1	.707107
43x4	1	1	1	1	.707107
43x4	2	1	1	1	.707107
43x4	3	3	2	2	.707107
43x4	4	1	2	3	1.224745
43x4	5	2	2	3	1.224745
167x192	1	3	1	1	.707107
167x192	2	2	1	1	.707107
167x192	3	3	1	1	.707107
167x192	4	3	2	3	1.224745
167x192	5	3	2	2	.707107
167x367	1	1	1	1	.707107
167x367	2	1	1	1	.707107

167x367	3	2	2	3	1.224745
167x367	4	3	1	2	.707107
167x367	5	2	1	1	.707107
43x466	1	2	1	1	.707107
43x466	2	2	1	1	.707107
43x466	3	3	2	2	.707107
43x466	4	2	1	1	.707107
43x466	5	1	1	1	.707107
167x4	1	1	1	1	.707107
167x4	2	2	1	1	.707107
167x4	3	3	2	2	.707107
167x4	4	2	2	2	.707107
167x4	5	2	2	2	.707107
223x43	1	2	2	2	.707107
223x43	2	3	2	2	.707107
223x43	3	3	2	2	.707107
223x43	4	1	1	1	.707107
223x43	5	2	1	1	.707107
4x167	1	2	2	2	.707107
4x167	2	3	1	1	.707107
4x167	3	2	2	2	.707107
4x167	4	2	2	2	.707107
4x167	5	2	1	1	.707107
167x223	1	3	2	2	.707107
167x223	2	1	1	1	.707107
167x223	3	3	3	2	.707107
167x223	4	1	2	2	.707107
167x223	5	1	1	1	.707107
167x343	1	2	1	1	.707107
167x343	2	2	1	1	.707107
167x343	3	2	1	1	.707107
167x343	4	2	1	1	.707107
167x343	5	3	2	2	.707107
43x343	1	2	2	2	.707107
43x343	2	2	1	1	.707107
43x343	3	2	1	1	.707107
43x343	4	2	3	2	.707107
43x343	5	1	1	1	.707107
43x167	1	3	1	1	.707107
43x167	2	2	2	2	.707107
43x167	3	2	2	1	.707107
43x167	4	1	1	1	.707107
43x167	5	2	1	1	.707107
167x466	1	1	1	1	.707107
167x466	2	3	2	2	.707107
167x466	3	2	1	1	.707107
167x466	4	1	1	1	.707107
167x466	5	3	1	1	.707107
192x167	1	1	1	1	.707107
192x167	2	2	1	1	.707107
192x167	3	2	1	1	.707107
192x167	4	1	2	2	.707107
192x167	5	2	1	1	.707107
4x43	1	2	1	1	.707107
4x43	2	3	2	2	.707107
4x43	3	1	3	3	7.245688
4x43	4	1	1	1	.707107
4x43	5	1	1	1	.707107
223x167	1	2	2	1	.707107
223x167	2	3	1	1	.707107
223x167	3	3	1	1	.707107
223x167	4	3	1	1	.707107
223x167	5	3	2	2	.707107
4x466	1	2	1	1	.707107
4x466	2	1	1	1	.707107

4x466	3	1	2	2	.707107
4x466	4	2	1	2	.707107
4x466	5	1	2	2	.707107
111x466	1	1	1	1	.707107
111x466	2	2	1	1	.707107
111x466	3	1	1	1	.707107
111x466	4	2	1	1	.707107
111x466	5	1	1	1	.707107
167x111	1	2	2	1	.707107
167x111	2	2	2	1	.707107
167x111	3	2	1	1	.707107
167x111	4	2	1	1	.707107
167x111	5	3	1	1	.707107
167x304	1	1	1	1	.707107
167x304	2	1	1	1	.707107
167x304	3	2	1	1	.707107
167x304	4	1	1	1	.707107
167x304	5	1	1	1	.707107
167x43	1	2	1	1	.707107
167x43	2	3	1	1	.707107
167x43	3	3	1	1	.707107
167x43	4	3	1	1	.707107
167x43	5	3	1	1	.707107
223x466	1	2	1	1	.707107
223x466	2	2	2	1	.707107
223x466	3	2	1	1	.707107
223x466	4	2	1	1	.707107
223x466	5	1	1	1	.707107
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43x192	2	1	1	1	.707107
43x192	4	2	1	1	.707107
43x192	5	2	1	1	.707107
43x223	1	1	1	1	.707107
43x223	2	1	1	1	.707107
43x223	3	1	1	1	.707107
43x223	4	2	1	1	.707107
43x223	5	1	1	1	.707107
192x466	1	1	1	1	.707107
192x466	2	2	1	1	.707107
192x466	3	1	1	1	.707107
192x466	4	2	1	1	.707107
192x466	5	2	1	1	.707107
111x43	1	2	1	1	.707107
111x43	2	1	1	1	.707107
111x43	3	1	1	1	.707107
111x43	4	2	1	1	.707107
111x43	5	1	1	1	.707107
223x304	1	2	1	1	.707107
223x304	2	2	1	1	.707107
223x304	3	2	1	1	.707107
223x304	4	3	2	1	.707107
223x304	5	3	1	1	.707107
223x343	1	1	1	1	.707107
223x343	2	1	1	1	.707107
223x343	3	1	1	1	.707107
223x343	4	1	1	1	.707107
223x343	5	1	1	1	.707107
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111x343	3	1	1	1	.707107
111x343	4	1	1	1	.707107
111x343	5	2	1	1	.707107
43x111	1	2	1	1	.707107
43x111	2	2	2	1	.707107
43x111	3	2	1	1	.707107

43x111	4	2	1	1	.707107
43x111	5	2	1	1	.707107
192x43	1	1	1	1	.707107
192x43	2	1	1	1	.707107
192x43	3	1	1	1	.707107
192x43	4	1	1	1	.707107
192x43	5	1	1	1	.707107
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192x111	2	2	1	1	.707107
192x111	3	2	1	1	.707107
192x111	4	1	1	1	.707107
192x111	5	2	1	1	.707107
223x111	1	2	1	1	.707107
223x111	2	2	1	1	.707107
223x111	3	1	1	1	.707107
223x111	4	1	1	1	.707107
223x111	5	2	1	1	.707107
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111x304	3	2	1	1	.707107
111x304	4	1	1	1	.707107
111x304	5	3	1	1	.707107
111x167	1	1	1	1	.707107
111x167	2	3	1	1	.707107
111x167	3	2	1	1	.707107
111x167	4	2	1	1	.707107
111x167	5	2	1	1	.707107
43x304	1	1	1	1	.707107
43x304	2	2	1	1	.707107
43x304	3	2	1	1	.707107
43x304	4	1	1	1	.707107
43x304	5	3	1	1	.707107
304x223	1	2	1	1	.707107
304x466	2	1	1	1	.707107
343x4	3	2	1	1	.707107
343x4	1	1	1	1	.707107
304x43	2	2	1	1	.707107
466x4	3	1	1	1	.707107
466x367	1	3	1	1	.707107
466x4	1	2	1	1	.707107
304x343	3	1	1	1	.707107
367x43	3	1	1	1	.707107
304x167	4	2	1	1	.707107
466x304	1	1	1	1	.707107
304x343	4	1	1	1	.707107
304x223	3	2	1	1	.707107
304x4	5	1	1	1	.707107
367x466	1	2	1	1	.707107
466x111	2	1	1	1	.707107
466x367	2	1	1	1	.707107
304x111	4	2	1	1	.707107
343x111	4	2	1	1	.707107
304x167	3	1	1	1	.707107
343x43	3	1	1	1	.707107
343x466	4	1	1	1	.707107
304x43	4	3	1	1	.707107
304x466	4	2	1	1	.707107
466x223	2	1	1	1	.707107
304x223	5	1	1	1	.707107
367x223	5	1	1	1	.707107
466x343	2	1	1	1	.707107
343x466	1	1	1	1	.707107
343x304	5	1	1	1	.707107
304x111	5	1	1	1	.707107
343x466	2	1	1	1	.707107

343x304	3	1	1	1	.707107
466x43	5	2	1	1	.707107
304x466	5	1	1	1	.707107
343x223	1	1	1	1	.707107
343x466	5	3	1	1	.707107
304x367	5	1	1	1	.707107
304x367	2	1	1	1	.707107
304x167	1	3	1	1	.707107
466x367	5	3	1	1	.707107
466x111	5	1	1	1	.707107
304x367	3	2	1	1	.707107
343x304	2	1	1	1	.707107
367x167	4	1	1	1	.707107
343x367	4	1	1	1	.707107
304x111	1	1	1	1	.707107
304x223	4	3	1	1	.707107
343x4	2	1	1	1	.707107
304x111	3	2	1	1	.707107
367x304	4	1	1	1	.707107
304x4	2	1	1	1	.707107
343x466	3	2	1	1	.707107
343x167	1	1	1	1	.707107
304x466	1	1	1	1	.707107
466x167	1	3	1	1	.707107
367x43	2	1	1	1	.707107
304x343	2	1	1	1	.707107
343x223	3	2	1	1	.707107
343x43	4	1	1	1	.707107
466x4	2	1	1	1	.707107
466x43	4	1	1	1	.707107
466x167	5	3	1	1	.707107
466x43	3	1	1	1	.707107
367x167	1	1	1	1	.707107
367x304	5	1	1	1	.707107
466x43	2	1	1	1	.707107
304x43	1	1	1	1	.707107
367x466	5	1	1	1	.707107
466x304	5	1	1	1	.707107
304x343	1	1	1	1	.707107
466x343	3	1	1	1	.707107
466x304	4	2	1	1	.707107
343x223	2	2	1	1	.707107
367x111	3	2	1	1	.707107
466x192	4	3	1	1	.707107
466x192	5	1	1	1	.707107
304x466	3	2	1	1	.707107
343x167	4	2	1	1	.707107
466x223	4	1	1	1	.707107
367x43	5	1	1	1	.707107
367x111	5	1	1	1	.707107
466x367	3	1	1	1	.707107
343x223	5	1	1	1	.707107
343x304	4	1	1	1	.707107
466x43	1	1	1	1	.707107
466x304	2	1	1	1	.707107
343x167	5	1	1	1	.707107
367x223	1	1	1	1	.707107
367x4	5	2	1	1	.707107
343x192	1	1	1	1	.707107
367x343	2	1	1	1	.707107
304x167	5	3	1	1	.707107
466x167	2	1	1	1	.707107
304x367	4	2	1	1	.707107
343x192	2	1	1	1	.707107
466x343	1	2	1	1	.707107

367x343	5	1	1	1	.707107
466x167	3	3	1	1	.707107
367x192	1	1	1	1	.707107
466x192	1	1	1	1	.707107
367x466	3	1	1	1	.707107
304x43	3	2	1	1	.707107
466x192	3	3	1	1	.707107
466x223	1	2	1	1	.707107
343x223	4	1	1	1	.707107
304x192	1	2	1	1	.707107
304x192	5	2	1	1	.707107
466x111	4	1	1	1	.707107
304x343	5	1	1	1	.707107
343x192	4	1	1	1	.707107
466x343	5	1	1	1	.707107
343x304	1	2	1	1	.707107
304x167	2	1	1	1	.707107
466x167	4	2	1	1	.707107
466x223	5	3	1	1	.707107
304x192	2	3	1	1	.707107
367x466	2	1	1	1	.707107
466x223	3	2	1	1	.707107
466x111	3	3	1	1	.707107
343x43	2	1	1	1	.707107
304x4	3	2	2	2	.707107
466x4	4	1	2	2	.707107
466x4	5	3	3	2	.707107
304x192	3	2	2	2	.707107
304x43	5	2	2	2	.707107
466x192	2	3	3	2	.707107
304x223	2	3	2	2	.707107
343x111	2	1	2	2	.707107
343x43	5	1	2	2	.707107
343x43	1	2	2	2	.707107
343x167	2	3	2	2	.707107
343x111	1	2	2	2	.707107
466x367	4	2	2	2	.707107
343x4	5	1	2	2	.707107
466x304	3	1	2	2	.707107
343x192	5	2	2	2	.707107
343x111	3	2	2	3	4.527693
343x192	3	2	2	3	1.581139
304x111	2	1	2	2	.707107
343x4	4	2	2	3	2.915476
466x343	4	2	2	3	1.224745
343x167	3	3	2	3	1.581139
466x111	1	3	2	3	1.581139
343x367	3	1	2	3	3.240370
343x111	5	2	2	3	3.937004
343x367	5	1	2	3	2.549510
304x4	4	3	2	3	1.581139
343x367	1	1	2	3	1.224745
304x367	1	1	2	3	2.121320
343x367	2	1	2	3	1.581139
304x4	1	3	2	3	1.870829
304x192	4	2	2	3	1.870829
192x343	1	3	3	2	.707107
192x343	2	3	3	2	.707107
192x343	3	3	3	2	.707107
192x343	4	3	3	2	.707107
192x343	5	3	3	2	.707107
367x167	1	3	2	3	1.224745
367x304	1	1	2	3	1.581139
367x192	2	2	2	3	1.870829
367x223	2	1	2	3	1.224745

367x223	3	1	2	3	1.224745
367x4	3	3	2	3	2.345208
367x343	1	1	2	3	1.224745
367x167	5	2	2	3	1.870829
367x304	3	1	2	3	1.224745
367x192	4	2	2	2	.707107
367x43	1	1	2	3	2.915476
367x343	3	1	2	3	1.224745
367x4	2	2	3	3	6.204837
367x304	2	2	2	3	2.345208
367x4	4	2	2	3	3.937004
367x111	1	1	2	3	2.549510
367x223	4	1	2	3	2.121320
367x4	1	2	2	3	3.082207
367x192	3	2	3	3	5.958188
367x43	4	1	2	3	4.062019
367x111	4	2	2	3	1.870829
367x111	2	1	2	2	.707107
367x192	5	1	2	2	.707107
367x167	2	2	2	2	.707107
367x466	4	2	1	1	.707107
367x343	4	1	1	1	.707107
4x234	1	2	1	1	.707107
4x234	2	2	1	1	.707107
4x234	3	1	1	1	.707107
4x234	4	2	2	2	.707107
4x234	5	2	2	2	.707107
4x5	1	1	1	1	.707107
4x5	2	1	1	1	.707107
4x5	3	2	1	1	.707107
4x5	4	2	1	1	.707107
4x5	5	2	2	3	1.224745
192x5	1	2	1	1	.707107
192x5	2	2	1	1	.707107
192x5	3	3	1	1	.707107
192x5	4	2	1	1	.707107
192x5	5	1	2	2	.707107
111x234	1	1	1	1	.707107
111x234	2	2	1	1	.707107
111x234	3	1	1	1	.707107
111x234	4	1	1	1	.707107
111x234	5	1	1	1	.707107
223x234	1	2	1	2	.707107
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223x234	3	1	1	1	.707107
223x234	4	3	1	1	.707107
223x234	5	2	1	2	.707107
192x244	1	1	1	1	.707107
192x244	2	2	1	1	.707107
192x244	3	3	1	1	.707107
192x244	4	1	1	1	.707107
192x244	5	1	1	1	.707107
43x234	1	1	1	1	.707107
43x234	2	2	1	1	.707107
43x234	3	1	1	1	.707107
43x234	4	1	1	1	.707107
43x234	5	3	1	1	.707107
192x234	1	2	1	1	.707107
192x234	2	1	1	1	.707107
192x234	3	1	1	1	.707107
192x234	4	2	1	1	.707107
192x234	5	2	1	1	.707107
111x5	1	1	1	1	.707107
111x5	2	1	1	1	.707107
111x5	3	1	1	1	.707107

111x5	4	2	1	1	.707107
111x5	5	1	1	1	.707107
304x234	3	2	1	1	.707107
244x4	5	2	1	1	.707107
244x43	1	1	1	1	.707107
343x244	5	2	1	1	.707107
244x43	3	1	1	1	.707107
466x234	4	1	1	1	.707107
5x192	1	2	1	1	.707107
5x111	1	2	1	1	.707107
343x234	5	1	1	1	.707107
60x192	2	2	1	1	.707107
343x234	4	1	1	1	.707107
244x343	2	1	1	1	.707107
5x111	3	2	1	1	.707107
343x244	4	2	1	1	.707107
60x343	4	1	1	1	.707107
244x192	3	3	1	1	.707107
5x111	2	1	1	1	.707107
5x367	3	1	1	1	.707107
5x192	5	3	1	1	.707107
60x343	1	1	1	1	.707107
60x4	2	1	1	1	.707107
367x234	5	1	1	1	.707107
466x234	1	1	1	1	.707107
244x4	4	1	1	1	.707107
304x234	4	2	1	1	.707107
367x244	4	1	1	1	.707107
5x111	4	2	1	1	.707107
244x43	4	1	1	1	.707107
343x244	3	3	1	1	.707107
60x192	1	2	1	1	.707107
60x192	3	1	1	1	.707107
60x192	5	1	1	1	.707107
60x192	4	1	1	1	.707107
244x192	4	2	1	1	.707107
60x4	3	1	1	1	.707107
367x244	5	1	1	1	.707107
244x343	1	1	1	1	.707107
60x4	5	2	1	1	.707107
367x234	3	1	1	1	.707107
244x43	2	2	1	1	.707107
343x234	2	1	1	1	.707107
343x234	1	2	1	1	.707107
466x234	5	1	1	1	.707107
5x367	1	2	1	1	.707107
304x234	2	3	1	1	.707107
60x343	2	2	1	1	.707107
244x4	2	1	1	1	.707107
60x4	4	1	1	1	.707107
5x367	2	2	1	1	.707107
244x343	4	1	1	1	.707107
367x244	3	1	1	1	.707107
244x43	5	2	1	1	.707107
244x4	1	3	1	1	.707107
304x234	1	1	1	1	.707107
5x367	5	2	1	1	.707107
60x4	1	3	1	1	.707107
5x192	3	2	1	1	.707107
343x244	2	3	1	1	.707107
5x111	5	1	1	1	.707107
466x234	2	3	1	1	.707107
244x192	1	2	2	2	.707107
5x367	4	2	2	2	.707107
244x4	3	1	2	2	.707107

367x244	2	1	2	2	.707107
5x192	4	2	2	2	.707107
5x192	2	1	2	2	.707107
343x244	1	1	2	2	.707107
244x192	5	1	2	2	.707107
367x244	1	1	2	3	1.224745
343x234	3	2	2	3	3.535534
367x234	2	2	3	3	5.700877
244x343	5	3	2	3	3.082207
60x343	5	1	2	3	2.121320
367x234	4	3	3	3	4.062019
244x343	3	2	2	3	2.738613
60x343	3	2	2	3	3.535534
367x234	1	3	1	1	.707107
244x192	2	2	1	1	.707107
466x234	3	2	1	1	.707107
304x234	5	1	1	1	.707107
192x60	1	1	1	1	.707107
192x60	2	2	1	1	.707107
192x60	3	2	1	1	.707107
192x60	4	2	1	1	.707107
192x60	5	1	1	1	.707107
167x234	1	2	1	1	.707107
167x234	2	2	2	2	.707107
167x234	3	2	2	2	.707107
167x234	4	3	1	1	.707107
167x234	5	1	1	1	.707107

Cycle 2

Half-sib	Entry	Callus size	C. friability	C. color	# SE(transformed)
367x111(1)	1	3	F	clear	3.39116
367x111(1)	2	2	M	clear	.707107
367x111(1)	3	3	F	clear	1.22474
367x111(1)	4	3	M	cl/beige	.707107
367x111(1)	5	4	F	clear	.707107
367x111(1)	6	4	F	clear	.707107
367x111(1)	7	3	F	clear	.707107
343x192(3)	1	3	F	clear	.707107
343x192(3)	2	4	F	clear	.707107
343x192(3)	3	4	F	clear	5.14782
343x192(3)	4	3	F	clear	.707107
343x192(3)	5	3	F	clear	3.24037
343x192(3)	6	4	F	clear	3.53553
343x192(3)	7	4	F	clear	.707107
367x304(1)	1	4	M	cl/green	3.80789
367x304(1)	2	3	F	cl/green	2.73861
367x304(1)	3	3	F	clear	.707107
367x304(1)	4	4	F	cl/green	.707107
367x304(1)	5	4	F	clear	.707107
367x304(1)	6	3	F	clear	.707107
367x304(1)	7	3	F	clear	.707107
367x343(1)	1	4	F	clear	3.24037
367x343(1)	2	4	F	clear	1.22474
367x343(1)	3	4	F	clear	.707107
367x343(1)	4	3	M	clear	1.22474
367x343(1)	5	3	F	clear	3.08221
367x343(1)	6	4	F	clear	1.22474
367x343(1)	7	4	F	clear	.707107
43x367(3)	1	3	M	clear	.707107
43x367(3)	2	3	F	clear	.707107
43x367(3)	3	3	F	clear	.707107
43x367(3)	4	3	F	clear	.707107
43x367(3)	5	4	F	clear	1.22474

43x367(3)	6	3	F	clear	.707107
43x367(3)	7	3	F	clear	.707107
192x367(3)	1	3	M(few ce	cl/beige	.707107
192x367(3)	2	4	M	clear	1.22474
192x367(3)	3	4	F	clear	.707107
192x367(3)	4	4	F	clear	.707107
192x367(3)	5	3	F	clear	.707107
192x367(3)	6	4	F	cl/green	7.58288
192x367(3)	7	4	F	cl/green	.707107
367x167(3)	1	4	F	clear	.707107
367x167(3)	2	4	F	clear	.707107
367x167(3)	3	4	F	clear	3.08221
367x167(3)	4	3	M	clear	.707107
367x167(3)	5	3	F	clear	.707107
367x167(3)	6	4	F	cl/beige	1.87083
367x167(3)	7	4	F	clear	.707107
343x4(4)	1	4	F	clear	4.30116
343x4(4)	2	4	F	clear	1.22474
343x4(4)	3	3	F	clear	1.22474
343x4(4)	4	3	F	clear	2.12132
343x4(4)	5	4	F	clear	4.74342
343x4(4)	6	4	F	clear	.707107
343x4(4)	7	4	M	clear	2.54951
167x367(3)	1	5	F	clear	.707107
167x367(3)	2	3	F	clear	.707107
167x367(3)	3	4	F	clear	1.22474
167x367(3)	4	3	F	clear	.707107
167x367(3)	5	4	F	clear	3.39116
167x367(3)	6	4	F	clear	.707107
167x367(3)	7	4	F	clear	.707107
111x367(3)	1	3	F	clear	.707107
111x367(3)	2	3	F	cl/green	1.22474
111x367(3)	3	4	F	clear	.707107
111x367(3)	4	3	M	cl/beige	3.39116
111x367(3)	5	4	F	clear	.707107
111x367(3)	6	4	F	clear	3.93700
111x367(3)	7	3	M	clear	1.22474
4x111(3)	1	3	F	clear	1.87083
4x111(3)	2	3	M	cl/beige	.707107
4x111(3)	3	3	F	cl/green	1.22474
4x111(3)	4	4	M	cl/beige	.707107
4x111(3)	5	4	F	clear	.707107
4x111(3)	6	4	F	clear	3.39116
4x111(3)	7	4	F	clear	4.63681
343x167(3)	1	3	M	clear	2.34521
343x167(3)	2	3	F	cl/beige	.707107
343x167(3)	3	3	M	clear	3.53553
343x167(3)	4	4	M	cl/red	.707107
343x167(3)	5	4	M	cl/beige	.707107
343x167(3)	6	4	F	clear	.707107
343x167(3)	7	4	F	very cle	2.34521
4x304(5)	1	4	M	clear	.707107
4x304(5)	2	4	F	very cle	.707107
4x304(5)	3	2	F(few ce	clear	.707107
4x304(5)	4	3	M(few ce	clear	5.52268
4x304(5)	5	4	M	clear	.707107
4x304(5)	6	4	F	clear	.707107
4x304(5)	7	3	H	yell/gre	.707107
367x304(2)	1	4	M	cl/red	.707107
367x304(2)	2	4	M	clear	.707107
367x304(2)	3	4	M	clear	.707107
367x304(2)	4	4	F	clear	.707107
367x304(2)	5	4	F	cl/beige	1.22474
367x304(2)	6	4	F	clear	.707107
367x304(2)	7	3	F	clear	.707107

4x5(5)	1	3	F	clear	.707107
4x5(5)	2	3	F	clear	2.12132
4x5(5)	3	3	F	clear	2.34521
4x5(5)	4	3	M	clear	5.78792
4x5(5)	5	3	F	clear	.707107
4x5(5)	6	4	F	clear	2.54951
4x5(5)	7	3	F	clear	1.22474
367x223(4)	1	4	M	beige	.707107
367x223(4)	2	4	M	beige	.707107
367x223(4)	3	3	M	cl/beige	.707107
367x223(4)	4	4	M	beige	.707107
367x223(4)	5	3	M	cl/beige	.707107
367x223(4)	6	5	F	cl/beige	3.80789
367x223(4)	7	3	M	beige	.707107
4x111(5)	1	4	F	clear	3.08221
4x111(5)	2	4	F	cl/beige	2.34521
4x111(5)	3	4	F	clear	.707107
4x111(5)	4	3	F	clear	2.34521
4x111(5)	5	3	F	clear	2.91548
4x111(5)	6	3	F	clear	5.78792
4x111(5)	7	4	F	clear	1.22474
4x223(5)	1	3	M	beige/re	.707107
4x223(5)	2	3	F	clear	4.41588
4x223(5)	3	3	F	clear	.707107
4x223(5)	4	3	F	cl/red	7.38241
4x223(5)	5	3	F	cl/beige	.707107
4x223(5)	6	3	F	cl/beige	3.24037
4x223(5)	7	3	M	cl/beige	1.87083
111x223(2)	1	4	F	clear	.707107
111x223(2)	2	3	M	cl/beige	.707107
111x223(2)	3	3	F	very cle	3.80789
111x223(2)	4	4	M	bei/red	2.12132
111x223(2)	6	4	F	beige	.707107
111x223(2)	7	4	F	clear	.707107
4x367(5)	1	4	F	cl/beige	4.74342
4x367(5)	2	4	F	cl/red	6.96419
4x367(5)	3	3	F	cl/beige	6.44205
4x367(5)	4	4	M	beige	.707107
4x367(5)	5	4	F	clear	4.52769
4x367(5)	6	3	F	beige	.707107
4x367(5)	7	3	M	beige	1.58114
4x192(2)	1	4	F	clear	.707107
4x192(2)	2	3	M	cl/beige	4.74342
4x192(2)	3	4	M	bei/gree	.707107
4x192(2)	4	4	F	clear	.707107
4x192(2)	5	4	F	very cle	6.12372
4x192(2)	6	4	F	cl/beige	1.22474
4x192(2)	7	4	F	beige	.707107
223x367(1)	1	3	F	beige	.707107
223x367(1)	2	3	F	beige	.707107
223x367(1)	3	3	M	beige	.707107
223x367(1)	4	3	M	beige	.707107
223x367(1)	5	3	F	clear	.707107
223x367(1)	6	3	F	clear	2.34521
223x367(1)	7	3	F	cl/red	.707107
367x4(4)	1	3	M	beige	.707107
367x4(4)	2	4	F	clear	6.67083
367x4(4)	3	3	F	beige	.707107
367x4(4)	4	4	F	cl/beige	2.12132
367x4(4)	5	5	M	cl/beige	2.54951
367x4(4)	6	3	F	cl/beige	.707107
367x4(4)	7	3	M	beige	.707107
304x192(4)	1	3	F	beige	3.93700
304x192(4)	2	4	M	beige	.707107
304x192(4)	3	4	M	bei/brow	.707107

304x192(4)	4	3	M	beige	.707107
304x192(4)	5	4	F	beige	.707107
304x192(4)	6	4	F	bei/brow	2.54951
304x192(4)	7	3	H	cl/beige	.707107
367x43(1)	1	3	F	clear	5.70088
367x43(1)	2	4	F	clear	.707107
367x43(1)	3	4	M	cl/beige	.707107
367x43(1)	4	3	F	clear	.707107
367x43(1)	5	3	F	cl/green	.707107
367x43(1)	6	3	F	beige	.707107
367x43(1)	7	4	F	cl/beige	5.04975
244x343(5)	1	4	M	clear	.707107
244x343(5)	2	4	M	clear	.707107
244x343(5)	3	4	F	beige	.707107
244x343(5)	4	3	F	clear	.707107
244x343(5)	5	3	F	beige	.707107
244x343(5)	6	5	M	beige	.707107
244x343(5)	7	4	F	bei/brow	3.53553
192x4(5)	1	4	F	clear	.707107
192x4(5)	2	4	F	very cle	.707107
192x4(5)	3	4	F	very cle	.707107
192x4(5)	4	4	M	beige	1.58114
192x4(5)	5	3	F	cl/beige	2.91548
192x4(5)	6	3	F	cl/beige	2.91548
192x4(5)	7	4	F	cl/beige	2.12132
343x111(5)	1	3	F	clear	.707107
343x111(5)	2	3	F	beige	.707107
343x111(5)	3	4	F	clear	2.54951
343x111(5)	4	4	F	beige	.707107
343x111(5)	5	4	F	cl/beige	4.74342
343x111(5)	6	3	F	cl/beige	.707107
343x111(5)	7	4	M	clear	.707107
367x192(2)	1	3	F	bei/brow	.707107
367x192(2)	2	4	F	very cle	.707107
367x192(2)	3	3	F	bei/brow	4.06202
367x192(2)	4	4	F	cl/beige	.707107
367x192(2)	5	3	F	bei/brow	.707107
367x192(2)	6	4	F	cl/beige	4.18330
367x192(2)	7	3	F	clear	.707107
304x4(1)	1	4	F	clear	4.06202
304x4(1)	2	4	F	beige	.707107
304x4(1)	3	4	M	cl/bei/r	6.20484
304x4(1)	4	4	F	cl/beige	4.41588
304x4(1)	5	4	F	clear	.707107
304x4(1)	6	4	F	cl/beige	.707107
304x4(1)	7	4	F	clear	1.22474
60x343(3)	1	3	M	beige	1.87083
60x343(3)	2	3	M	cl/bige	3.67423
60x343(3)	3	3	M	clear	2.12132
60x343(3)	4	4	F	clear	.707107
60x343(3)	5	3	F	clear	4.84768
60x343(3)	6	4	M	cl/red	2.91548
60x343(3)	7	4	M	cl/green	.707107
192x4(4)	1	4	F	very cle	2.54951
192x4(4)	2	4	F	very cle	6.89202
192x4(4)	3	3	M	beige	.707107
192x4(4)	4	3	F	clear	.707107
192x4(4)	5	3	F	clear	9.02774
192x4(4)	6	4	F	cl/beige	.707107
192x4(4)	7	3	F	cl/red	3.93700
343x367(1)	1	4	F	bei/brow	.707107
343x367(1)	2	4	M	beige	.707107
343x367(1)	3	3	M	cl/beige	.707107
343x367(1)	4	4	M	beige	.707107
343x367(1)	5	3	M	cl/beige	2.91548

343x367(1)	6	4	M	beige	2.54951
343x367(1)	7	3	M	cl/beige	.707107
304x4(4)	1	3	F	cl/red	.707107
304x4(4)	2	4	F	cl/beige	2.73861
304x4(4)	3	3	F	clear	.707107
304x4(4)	4	4	F	clear	.707107
304x4(4)	5	3	F	cl/red	3.80789
304x4(4)	6	3	F	clear	.707107
304x4(4)	7	3	F	clear	.707107
43x367(2)	1	3	F	cl/beige	3.39116
43x367(2)	2	3	F	beige	.707107
43x367(2)	3	3	F	clear	.707107
43x367(2)	4	4	F	beige	.707107
43x367(2)	5	4	F	beige	.707107
43x367(2)	6	3	M	beige	.707107
43x367(2)	7	3	F	cl/beige	.707107
367x244(1)	1	3	F	cl/beige	.707107
367x244(1)	2	3	F	beige	.707107
367x244(1)	3	3	F	cl/beige	.707107
367x244(1)	4	3	M	beige	.707107
367x244(1)	5	3	M	beige	.707107
367x244(1)	6	4	F	cl/beige	3.67423
367x244(1)	7	4	F	beige	.707107
367x43(4)	1	1	M	beige	.707107
367x43(4)	2	4	M	clear	.707107
367x43(4)	3	3	F	clear	7.17635
367x43(4)	4	5	M	cl/beige	.707107
367x43(4)	5	4	F	clear	.707107
367x43(4)	6	4	F	cl/beige	.707107
367x43(4)	7	4	M	bei/red	.707107
4x343(3)	1	4	M	clear	.707107
4x343(3)	2	4	M	beige	.707107
4x343(3)	3	4	M	beige	.707107
4x343(3)	4	4	F	beige	.707107
4x343(3)	5	4	M	beige	.707107
4x343(3)	6	4	M	bei/gree	.707107
4x343(3)	7	4	M	beige	.707107
367x234(2)	1	3	F	beige	.707107
367x234(2)	2	3	F	cl/beige	2.12132
367x234(2)	3	3	F	beige	.707107
367x234(2)	4	4	F	cl/beige	.707107
367x234(2)	5	4	F	cl/green	.707107
367x234(2)	6	3	F	cl/beige	.707107
367x234(2)	7	3	F	beige	.707107
43x4(5)	1	4	M	beige	1.87083
43x4(5)	2	4	F	cl/green	1.58114
43x4(5)	3	5	F	cl/beige	.707107
43x4(5)	4	3	F	beige	4.52769
43x4(5)	5	4	M	beige	.707107
43x4(5)	6	4	F	cl/red	.707107
43x4(5)	7	3	F	beige	.707107
367x4(2)	1	3	M	cl/beige	.707107
367x4(2)	2	4	F	clear	2.73861
367x4(2)	3	4	F	cl/red	.707107
367x4(2)	4	4	M	clear	3.53553
367x4(2)	5	4	F	cl/beige	6.28490
367x4(2)	6	4	M	cl/beige	.707107
367x4(2)	7	3	M	cl/bei	.707107
367x111(4)	1	4	F	beige	2.34521
367x111(4)	2	4	F	clear	3.80789
367x111(4)	3	3	F	clear	.707107
367x111(4)	4	4	F	clear	.707107
367x111(4)	5	4	F	clear	1.22474
367x111(4)	6	4	F	clear	.707107
367x111(4)	7	4	F	clear	.707107

4x192(4)	1	3	F	cl/red	.707107
4x192(4)	2	4	F	cl/yello	2.12132
4x192(4)	3	3	F	bei/red	4.74342
4x192(4)	4	4	F	cl/red	6.04152
4x192(4)	5	3	M	cl/beige	.707107
4x192(4)	6	4	F	cl/red	3.67423
4x192(4)	7	4	F	cl/beige	.707107
466x343(4)	1	4	F	clear	.707107
466x343(4)	2	4	F	clear	.707107
466x343(4)	3	3	F	cl/beige	1.58114
466x343(4)	4	3	F	cl/beige	.707107
466x343(4)	5	3	F	clear	.707107
466x343(4)	6	4	F	clear	2.91547
466x343(4)	7	3	M	beige	.707107
367x304(3)	1	4	F	clear	4.18330
367x304(3)	2	4	F	beige	.707107
367x304(3)	3	4	F	beige	2.54951
367x304(3)	4	3	F	clear	.707107
367x304(3)	5	3	F	clear	.707107
367x304(3)	6	3	F	clear	.707107
367x304(3)	7	4	M	clear	5.43139
343x304(3)	1	3	M	beige1	2.73861
343x304(3)	2	3	F	clear	.707107
343x304(3)	3	3	F	clear	1.87083
343x304(3)	4	3	M	beige1	1.22474
343x304(3)	5	3	M	beige1	.707107
343x304(3)	6	3	M	beige1	.707107
343x304(3)	7	3	M	beige1	.707107
192x367(5)	1	2	F	clear	.707107
192x367(5)	2	3	F	cl/beige	.707107
192x367(5)	3	4	F	clear	7.58288
192x367(5)	4	4	F	clear	7.38241
192x367(5)	5	5	M	clear	4.52769
192x367(5)	6	4	F	clear	.707107
192x367(5)	7	3	F	cl/red	.707107
466x111(1)	1	3	F	clear	.707107
466x111(1)	2	4	F	beige1	.707107
466x111(1)	3	3	F	cl/beige	.707107
466x111(1)	4	4	F	clear	2.34521
466x111(1)	5	4	M	clear	.707107
466x111(1)	6	4	F	cl/red	.707107
466x111(1)	7	4	M	cl/beige	.707107
43x4(4)	1	4	F	clear	.707107
43x4(4)	2	4	F	bei/red1	.707107
43x4(4)	3	4	F	cl/green	2.54951
43x4(4)	4	3	M	bei/brow	.707107
43x4(4)	5	3	F	beige1	.707107
43x4(4)	6	4	M	beige1	.707107
43x4(4)	7	3	M	beige1	.707107
111x192(4)	1	3	M	clear	4.30116
111x192(4)	2	3	F	cl/red	.707107
111x192(4)	3	4	F	cl/red	.707107
111x192(4)	4	3	F	clear	4.18330
111x192(4)	5	3	F	clear	2.73861
111x192(4)	6	3	F	cl/red	.707107
111x192(4)	7	4	F	cl/red	.707107
4x192(1)	1	3	F	clear	.707107
4x192(1)	2	3	F	clear	.707107
4x192(1)	3	4	F	very cle	2.12132
4x192(1)	4	4	F	very cle	4.30116
4x192(1)	5	4	F	very cle	1.58114
4x192(1)	6	3	M	clear	5.78792
4x192(1)	7	3	F	clear	.707107
4x343(5)	1	4	M	beige1	.707107
4x343(5)	2	3	M	bei/brow	.707107

4x343(5)	3	4	F	clear	.707107
4x343(5)	4	3	M	cl/beige	.707107
4x343(5)	5	4	M	cl/red/g	4.18330
4x343(5)	6	3	M	clear	.707107
4x343(5)	7	4	M	cl/beige	.707107
367x192(3)	1	4	F	clear	2.34521
367x192(3)	2	3	F	clear	4.63681
367x192(3)	3	4	F	clear	2.73861
367x192(3)	4	3	F	clear	3.08221
367x192(3)	5	4	F	clear	.707107
367x192(3)	6	3	F	very cle	.707107
367x192(3)	7	4	F	beige1	3.08221
223x192(3)	1	4	F	cl/red	.707107
223x192(3)	2	4	F	cl/beige	.707107
223x192(3)	3	3	F	clear	3.08221
223x192(3)	4	4	F	clear	.707107
223x192(3)	5	4	F	cl/beige	.707107
223x192(3)	6	4	F	clear	7.38241
223x192(3)	7	4	M	cl/beige	.707107
343x367(5)	1	4	M	cl/beige	.707107
343x367(5)	2	4	M	cl/beige	.707107
343x367(5)	3	3	M	cl/yell1	.707107
343x367(5)	4	4	M	clear	2.73861
343x367(5)	5	4	F	beige1	1.22474
343x367(5)	6	3	F	clear	4.74342
343x367(5)	7	4	F	clear	5.04975
111x367(1)	1	4	M	cl/beige	.707107
111x367(1)	2	3	M	cl/beige	2.91548
111x367(1)	3	3	M	clear	3.93700
111x367(1)	4	3	F	clear	1.87083
111x367(1)	5	4	F	beige1	.707107
111x367(1)	6	4	M	beige1	.707107
111x367(1)	7	3	M	beige1	1.58114
223x4(4)	1	4	F	clear	.707107
223x4(4)	2	4	F	cl/beige	1.22474
223x4(4)	3	3	F	clear	1.22474
223x4(4)	4	3	F	cl/beige	4.06202
223x4(4)	5	3	F	clear	1.22474
223x4(4)	6	4	F	clear	.707107
223x4(4)	7	4	F	clear	.707107
343x111(3)	1	3	F	clear	.707107
343x111(3)	2	4	F	clear	1.87083
343x111(3)	3	3	F	clear	.707107
343x111(3)	4	4	F	clear	.707107
343x111(3)	5	4	F	clear	.707107
343x111(3)	6	4	F	clear	.707107
343x111(3)	7	4	F	clear	.707107
223x4(2)	1	3	F	bei/red1	.707107
223x4(2)	2	3	M	beige1	.707107
223x4(2)	3	4	F	cl/red	2.34521
223x4(2)	4	4	F	clear	3.39116
223x4(2)	5	3	F	beige1	1.87083
223x4(2)	6	4	F	cl/red	3.53553
223x4(2)	7	4	F	clear	.707107
367x4(1)	1	4	M	cl/red	4.63681
367x4(1)	2	4	F	clear	3.24037
367x4(1)	3	4	F	clear	3.67423
367x4(1)	4	4	F	clear	.707107
367x4(1)	5	4	F	cl/red	3.67423
367x4(1)	6	4	F	clear	.707107
367x4(1)	7	4	F	clear	.707107
367x4(3)	1	3	M	cl/red	.707107
367x4(3)	2	3	M	clear	2.12132
367x4(3)	3	3	M	cl/green	.707107
367x4(3)	4	3	M	cl/red	.707107

367x4(3)	5	4	M	red/cl/g	.707107
367x4(3)	6	3	M	cl/red	1.87083
367x4(3)	7	3	M	clear	.707107
367x223(2)	1	3	F	clear	6.36396
367x223(2)	2	3	M	beige	.707107
367x223(2)	3	4	F	clear	7.58287
367x223(2)	4	3	M	beige	.707107
367x223(2)	5	3	F	clear	3.93700
367x223(2)	6	2	F	clear	.707107
367x223(2)	7	4	M	beige	.707107
4x43(3)	1	3	F	beige	5.14781
4x43(3)	2	3	F	beige	5.33854
4x43(3)	3	3	F	clear	8.33667
4x43(3)	4	3	F	beige	5.14781
4x43(3)	5	3	F	clear	2.54951
4x43(3)	6	3	M	beige	.707107
4x43(3)	7	4	F	clear	.707107
192x367(1)	1	4	F	clear	4.94975
192x367(1)	2	4	F	cl-beige	.707107
192x367(1)	3	4	F	clear	3.53553
192x367(1)	4	4	F	very cle	.707107
192x367(1)	5	4	F	clear	3.93700
192x367(1)	6	4	F	clear	.707107
192x367(1)	7	3	F	clear	6.59545
367x223(3)	1	3	F	cl-beige	.707107
367x223(3)	2	4	F	clear	7.90569
367x223(3)	3	4	M	beige	.707107
367x223(3)	4	3	F	cl-beige	3.93700
367x223(3)	5	3	F	cl-beige	.707107
367x223(3)	6	3	M	cl-beige	1.22474
367x223(3)	7	4	M	beige	.707107
192x367(4)	1	3	M	clear	.707107
192x367(4)	2	1	H	brown	.707107
192x367(4)	3	3	M	cl-beige	.707107
192x367(4)	4	3	M	cl-beige	3.39116
192x367(4)	5	4	F	bei-red	.707107
192x367(4)	6	2	F	cl-brown	.707107
192x367(4)	7	4	F	bei-red	.707107
167x192(4)	1	3	F	clear	3.39116
167x192(4)	2	3	F	cl-red	3.24037
167x192(4)	3	4	F	red	1.58114
167x192(4)	4	3	F	cl-red	3.80789
167x192(4)	5	3	F	clear	2.91547
167x192(4)	6	4	M	cl-red	.707107
167x192(4)	7	3	F	clear	.707107
111x4(5)	1	3	M	clear	.707107
111x4(5)	2	3	F	clear	.707107
111x4(5)	3	3	F	clear	.707107
111x4(5)	4	2	M	clear	.707107
111x4(5)	5	3	F	clear	.707107
111x4(5)	6	3	F	cl-green	6.96419
111x4(5)	7	3	F	clear	3.67423
367x343(3)	1	3	M	clear	5.95820
367x343(3)	2	4	F	clear	.707107
367x343(3)	3	3	M	clear	.707107
367x343(3)	4	4	M	clear	.707107
367x343(3)	5	3	F	clear	2.12132
367x343(3)	6	3	M	clear	.707107
367x343(3)	7	3	F	clear	.707107
192x367(2)	1	3	M	clear	1.22474
192x367(2)	2	3	F	cl-red	1.58114
192x367(2)	3	3	M	clear	1.22474
192x367(2)	4	3	M	clear	.707107
192x367(2)	5	4	F	clear	.707107
192x367(2)	6	3	F	clear	1.58114

192x367(2)	7	2	H	clear	.707107
343x367(3)	1	4	M	cl-green	.707107
343x367(3)	2	3	M	cl-green	.707107
343x367(3)	3	4	M	clear	.707107
343x367(3)	4	3	M	clear	.707107
343x367(3)	5	3	F	cl-red	.707107
343x367(3)	6	3	F	clear	4.63681
343x367(3)	7	3	F	clear	1.87083
244x343(3)	1	4	F	clear	5.24404
244x343(3)	2	3	F	clear	.707107
244x343(3)	3	3	F	cl-red	.707107
244x343(3)	4	3	M	cl-green	.707107
244x343(3)	5	3	M	clear	1.22474
244x343(3)	6	4	M	clear	.707107
244x343(3)	7	3	M	clear	.707107
343x367(2)	1	3	F	clear	3.39116
343x367(2)	2	3	F	clear	.707107
343x367(2)	3	3	M	clear	.707107
343x367(2)	4	3	F	clear	2.12132
343x367(2)	5	3	F	clear	.707107
343x367(2)	6	3	F	clear	3.53553
343X367(2)	7	3	M	clear	.707107

Comparative study

Cycle	Genotype	Entry	Rep.	C. size	C. friability	C. color	# SE(transformed)
0	FL77	150	1	1	f	clear	.707107
0	FL77	65	1	2	f	clear	.707107
0	FL77	69	1	2	h	bei/brow	.707107
0	FL77	37	1	2	m	bei/brow	.707107
0	FL77	5	1	2	m	cl/beige	.707107
0	FL77	103	1	3	h	beige	.707107
0	FL77	10	1	3	f	bei/brow	.707107
0	FL77	61	1	3	m	beige	.707107
0	FL77	68	1	3	f	clear	.707107
0	FL77	50	1	3	m	cl/brown	.707107
0	FL77	45	1	3	f	clear	.707107
0	FL77	38	1	3	f	clear	.707107
0	FL77	158	1	3	f	clear	.707107
0	FL77	20	1	3	f	clear	1.224745
0	FL77	153	1	3	f	clear	.707107
0	FL77	104	1	4	f	clear	.707107
0	FL77	9	1	4	m	cl/beige	.707107
0	FL77	66	1	4	f	cl/beige	.707107
0	FL77	2	1	4	m	cl/beige	.707107
0	FL77	102	1	4	f	clear	.707107
0	FL77	46	1	4	m	cl/beige	.707107
0	FL77	64	1	4	f	cl/beige	.707107
0	FL77	1	1	4	m	beige	.707107
0	FL77	24	1	4	f	clear	.707107
0	FL77	148	1	4	f	clear	.707107
0	FL77	47	1	4	m	cl/beige	.707107
0	FL77	22	1	4	m	cl/beige	.707107
0	FL77	109	1	4	m	cl/beige	.707107
0	FL77	159	1	4	f	clear	.707107
0	FL77	67	1	4	m	beige	.707107
0	FL77	12	1	4	h	beige	.707107
0	FL77	11	1	4	f	cl/beige	.707107
0	FL77	147	1	4	m	bei/brow	.707107
0	FL77	125	1	4	f	clear	.707107
0	FL77	126	1	4	m	cl/beige	.707107
0	FL77	149	1	5	m	cl/beige	.707107
0	FL77	23	1	5	f	cl/beige	.707107

0	FL77	154	1	5	f	cl/beige	.707107
0	FL77	6	1	5	f	clear	.707107
0	FL77	108	1	5	f	cl/beige	.707107
0	FL77	152	2	2	f	beige	.707107
0	FL77	56	2	2	f	beige	.707107
0	FL77	115	2	2	m	beige	.707107
0	FL77	25	2	3	f	cl/beige	.707107
0	FL77	14	2	3	f	cl/beige	.707107
0	FL77	13	2	3	f	clear	.707107
0	FL77	72	2	3	f	cl/beige	.707107
0	FL77	63	2	3	f	clear	.707107
0	FL77	112	2	3	f	beige	.707107
0	FL77	122	2	3	m	beige	.707107
0	FL77	3	2	3	f	clear	.707107
0	FL77	8	2	3	f	clear	.707107
0	FL77	27	2	3	f	clear	.707107
0	FL77	74	2	3	m	cl/beige	.707107
0	FL77	80	2	3	f	cl/beige	.707107
0	FL77	77	2	3	m	cl/beige	.707107
0	FL77	78	2	3	f	clear	.707107
0	FL77	30	2	3	h	beige	.707107
0	FL77	4	2	3	m	beige	.707107
0	FL77	121	2	3	f	cl/beige	.707107
0	FL77	15	2	4	f	beige	.707107
0	FL77	110	2	4	f	beige	.707107
0	FL77	40	2	4	m	beige	.707107
0	FL77	71	2	4	m	bei/red	.707107
0	FL77	73	2	4	f	bei/red	.707107
0	FL77	79	2	4	m	beige	.707107
0	FL77	157	2	4	f	cl/beige	.707107
0	FL77	111	2	4	f	beige	.707107
0	FL77	117	2	4	m	beige	2.12132
0	FL77	26	2	4	f	clear	.707107
0	FL77	28	2	4	f	cl/beige	.707107
0	FL77	7	2	4	m	beige	.707107
0	FL77	21	2	4	m	beige	.707107
0	FL77	29	2	4	f	clear	.707107
0	FL77	127	2	4	f	clear	.707107
0	FL77	105	2	5	f	cl/beige	.707107
0	FL77	39	2	5	f	cl/beige	.707107
0	FL77	70	2	5	f	clear	.707107
0	FL77	57	2	5	f	cl/red	.707107
0	FL77	123	2	5	f	clear	.707107
0	FL77	34	3	1	h	bei/brow	.707107
0	FL77	113	3	3	m	beige	.707107
0	FL77	83	3	3	m	beige	.707107
0	FL77	35	3	3	f	cl/beige	.707107
0	FL77	36	3	3	f	clear	.707107
0	FL77	48	3	3	f	cl/beige	.707107
0	FL77	160	3	3	m	beige	.707107
0	FL77	81	3	3	m	beige	.707107
0	FL77	82	3	3	m	beige	.707107
0	FL77	151	3	3	f	clear	.707107
0	FL77	156	3	3	f	clear	.707107
0	FL77	118	3	3	m	beige	.707107
0	FL77	87	3	3	f	beige	.707107
0	FL77	84	3	3	m	cl/beige	.707107
0	FL77	88	3	3	m	beige	.707107
0	FL77	16	3	3	m	beige	.707107
0	FL77	98	3	3	f	clear	.707107
0	FL77	93	3	3	m	beige	.707107
0	FL77	94	3	3	m	beige	.707107
0	FL77	135	3	3	f	clear	.707107
0	FL77	140	3	3	h	bei/brow	.707107
0	FL77	116	3	4	f	clear	.707107

0	FL77	18	3	4	h	bei/brow	.707107
0	FL77	33	3	4	f	cl/bei	4.18330
0	FL77	99	3	4	f	beige	.707107
0	FL77	41	3	4	m	cl/beige	.707107
0	FL77	120	3	4	h	beige	.707107
0	FL77	155	3	4	f	clear	.707107
0	FL77	32	3	4	f	cl/beige	.707107
0	FL77	31	3	4	f	cl/beige	.707107
0	FL77	114	3	4	f	cl/beige	.707107
0	FL77	19	3	4	m	cl/beige	.707107
0	FL77	17	3	4	f	clear	.707107
0	FL77	75	3	4	f	cl/red	3.24037
0	FL77	136	3	4	m	beige	.707107
0	FL77	76	3	4	m	beige	.707107
0	FL77	119	3	4	m	cl/beige	.707107
0	FL77	134	3	4	m	beige	.707107
0	FL77	138	3	4	m	cl/beige	.707107
0	FL77	139	3	5	m	beige	.707107
0	FL77	96	4	1	m	bronw	.707107
0	FL77	89	4	3	m	beige	.707107
0	FL77	97	4	3	f	clear	.707107
0	FL77	92	4	3	m	beige	.707107
0	FL77	90	4	3	f	beige	.707107
0	FL77	42	4	3	m	beige	.707107
0	FL77	137	4	3	m	beige	.707107
0	FL77	55	4	3	f	clear	.707107
0	FL77	58	4	3	f	clear	.707107
0	FL77	51	4	3	m	clear	.707107
0	FL77	85	4	3	f	clear	.707107
0	FL77	91	4	3	h	beige	.707107
0	FL77	144	4	3	m	beige	.707107
0	FL77	141	4	3	m	beige	.707107
0	FL77	95	4	3	m	beige	.707107
0	FL77	124	4	3	f	cl/beige	.707107
0	FL77	128	4	3	m	beige	.707107
0	FL77	60	4	3	m	beige	.707107
0	FL77	52	4	3	m	beige	.707107
0	FL77	132	4	4	f	clear	.707107
0	FL77	133	4	4	m	beige	.707107
0	FL77	86	4	4	f	clear	.707107
0	FL77	44	4	4	m	beige	.707107
0	FL77	43	4	4	f	clear	.707107
0	FL77	131	4	4	f	clear	.707107
0	FL77	101	4	4	f	cl/beige	.707107
0	FL77	54	4	4	f	clear	.707107
0	FL77	62	4	4	m	clear	.707107
0	FL77	106	4	4	f	clear	.707107
0	FL77	145	4	4	f	clear	.707107
0	FL77	146	4	4	f	clear	.707107
0	FL77	142	4	4	f	beige	.707107
0	FL77	143	4	4	m	beige	.707107
0	FL77	100	4	4	f	clear	.707107
0	FL77	130	4	4	f	clear	.707107
0	FL77	129	4	4	f	clear	.707107
0	FL77	53	4	4	m	beige	.707107
0	FL77	49	4	5	f	cl/red	.707107
0	FL77	107	4	5	f	clear	.707107
0	FL77	59	4	5	m	beige	.707107
1	43x4	1	1	2	f	beige	.707107
1	304x223	1	1	3	f	bei/brow	.707107
1	304x223	2	1	3	f	cl/beige	.707107
1	4x5	1	1	3	f	cl/beige	.707107
1	244x43	1	1	3	f	cl/beige	.707107
1	343x43	1	1	3	m	beige	.707107
1	343x192	1	1	3	f	cl/beige	1.870829

1	304x343	1	1	3	f	beige	.707107
1	304x343	2	1	3	f	cl/beige	.707107
1	466x43	1	1	3	f	cl/beige	.707107
1	304x367	1	1	3	f	cl/beige	.707107
1	304x367	2	1	3	f	cl/beige	3.24037
1	111x4	1	1	3	f	cl/beige	.707107
1	223x234	1	1	3	m	clear	.707107
1	466x4	1	1	3	f	clear	.707107
1	223x304	1	1	3	f	beige	.707107
1	223x192	1	1	3	f	cl/beige	.707107
1	4x234	1	1	3	f	clear	.707107
1	43x343	1	1	3	f	cl/beige	.707107
1	167x223	1	1	4	f	cl/beige	.707107
1	4x304	1	1	4	m	cl/beige	.707107
1	167x111	1	1	4	f	clear	1.224745
1	192x167	1	1	4	f	cl/beige	1.224745
1	223x192	2	1	4	f	cl/beige	.707107
1	343x167	1	1	4	m	bei/brow	.707107
1	466x43	2	1	4	m	clear	.707107
1	5x367	1	1	4	f	cl/beige	1.224745
1	244x43	2	1	4	m	beige	.707107
1	343x43	2	1	4	m	beige	.707107
1	4x234	2	1	4	f	clear	1.224745
1	167x111	2	1	4	m	clear	1.870829
1	4x304	1	1	4	f	cl/beige	.707107
1	343x367	1	1	4	f	clear	2.549510
1	343x244	1	1	4	m	clear	.707107
1	167x367	1	1	4	m	beige	.707107
1	4x223	1	1	4	f	clear	.707107
1	223x234	2	1	4	f	clear	.707107
1	43x304	1	1	4	m	beige	.707107
1	192x367	1	1	4	f	cl/beige	.707107
1	192x43	1	1	4	f	beige	.707107
1	43x367	1	2	3	m	cl/beige	.707107
1	43x367	2	2	3	m	cl/beige	.707107
1	111x43	1	2	3	f	cl/beige	.707107
1	111x43	2	2	3	f	cl/beige	.707107
1	192x43	2	2	3	f	clear	.707107
1	43x4	2	2	3	f	clear	1.224745
1	367x234	1	2	3	f	cl/beige	2.915476
1	223x4	1	2	3	f	clear	2.345208
1	466x367	1	2	3	m	beige	.707107
1	111x192	1	2	3	f	clear	.707107
1	367x223	2	2	3	f	beige	.707107
1	343x223	1	2	3	m	beige	.707107
1	192x223	1	2	4	f	cl/beige	.707107
1	192x223	2	2	4	f	clear	.707107
1	167x192	1	2	4	m	cl/beige	.707107
1	223x167	1	2	4	f	clear	.707107
1	43x192	1	2	4	f	cl/beige	.707107
1	343x367	2	2	4	f	clear	4.062019
1	466x367	2	2	4	f	clear	1.870829
1	223x4	2	2	4	f	beige	.707107
1	192x4	2	2	4	m	beige	.707107
1	367x466	1	2	4	f	clear	.707107
1	4x43	1	2	4	f	clear	1.870829
1	343x466	1	2	4	f	clear	.707107
1	4x5	2	2	4	m	beige	.707107
1	466x167	1	2	4	f	cl/beige	.707107
1	367x343	1	2	4	m	cl/beige	4.301163
1	5x367	2	2	4	m	beige	.707107
1	343x244	2	2	4	f	cl/beige	.707107
1	4x223	2	2	4	f	clear	.707107
1	167x4	1	2	4	m	cl/beige	.707107
1	304x234	1	2	4	f	cl/beige	.707107

1	43x192	2	2	4	f	clear	.707107
1	466x167	1	2	4	f	clear	.707107
1	367x223	1	2	4	m	clear	6.519202
1	43x304	2	2	4	m	beige	.707107
1	111x234	1	2	4	f	cl/beige	1.224745
1	43x234	1	2	4	m	cl/beige	.707107
1	43x234	2	2	4	m	beige	.707107
1	223x167	2	2	5	f	clear	.707107
1	466x111	1	3	2	m	bei/bron	.707107
1	367x43	1	3	3	m	cl/beige	.707107
1	111x304	1	3	3	m	beige	.707107
1	111x304	2	3	3	f	clear	.707107
1	4x1111	1	3	3	f	clear	.707107
1	4x111	2	3	3	f	clear	.707107
1	367x343	2	3	3	m	beige	1.224745
1	43x466	1	3	3	m	beige	.707107
1	304x43	1	3	3	f	bei/brow	.707107
1	111x5	1	3	3	f	clear	.707107
1	111x4	2	3	3	f	clear	.707107
1	367x167	1	3	3	f	clear	.707107
1	466x223	1	3	3	f	bei/brow	.707107
1	304x4	1	3	3	m	beige	.707107
1	343x4	1	3	3	m	beige	.707107
1	343x4	2	3	3	m	beige	.707107
1	111x466	1	3	3	f	clear	.707107
1	4x343	1	3	3	f	clear	.707107
1	304x111	1	3	3	f	beige	.707107
1	304x111	2	3	3	f	beige	.707107
1	466x4	2	3	4	f	clear	.707107
1	466x343	1	3	4	f	clear	.707107
1	367x43	2	3	4	f	beige	1.224745
1	4x43	2	3	4	m	beige	.707107
1	192x244	1	3	4	f	cl/beige	2.345208
1	466x343	2	3	4	m	beige	.707107
1	43x223	1	3	4	m	cl/beige	.707107
1	43x223	2	3	4	f	cl/beige	.707107
1	192x244	2	3	4	f	cl/beige	.707107
1	223x466	2	3	4	f	clear	.707107
1	192x367	2	3	4	m	beige	.707107
1	466x234	1	3	4	f	clear	.707107
1	466x223	2	3	4	f	beige	.707107
1	304x4	2	3	4	m	beige	.707107
1	466x111	2	3	4	f	clear	.707107
1	367x4	1	3	4	m	beige	.707107
1	192x304	1	3	4	f	clear	.707107
1	167x304	1	3	4	m	beige	.707107
1	4x343	2	3	4	f	clear	.707107
1	304x466	1	3	5	f	clear	.707107
1	4x466	1	4	3	f	clear	.707107
1	223x343	1	4	3	m	beige	.707107
1	192x466	1	4	3	f	clear	.707107
1	111x367	1	4	3	f	cl/beige	1.581139
1	304x192	1	4	3	m	beige	.707107
1	4x367	1	4	3	m	cl/beige	1.581139
1	466x192	1	4	3	m	beige	.707107
1	111x5	2	4	3	f	clear	.707107
1	304x167	1	4	3	f	clear	.707107
1	367x192	1	4	3	m	beige	.707107
1	223x343	2	4	3	m	beige	.707107
1	43x111	1	4	3	f	beige	.707107
1	4x192	1	4	3	f	cl/beige	.707107
1	367x304	1	4	3	m	beige	.707107
1	367x111	1	4	4	m	clear	.707107
1	167x466	1	4	4	f	clear	.707107
1	343x304	1	4	4	m	beige	.707107

1	167x343	1	4	4	f	beige	.707107
1	4x367	2	4	4	f	clear	6.819091
1	223x367	1	4	4	m	beige	.707107
1	4x192	2	4	4	f	clear	.707107
1	111x223	1	4	4	f	clear	.707107
1	192x5	2	4	4	m	cl/beige	.707107
1	111x343	1	4	4	f	cl/beige	.707107
1	223x43	1	4	4	m	beige	.707107
1	192x234	1	4	4	m	beige	.707107
1	111x167	1	4	4	m	beige	.707107
1	167x43	1	4	4	m	cl/beige	.707107
1	367x167	2	4	4	f	cl/beige	.707107
1	367x4	2	4	4	f	clear	1.870829
1	167x234	1	4	4	m	cl/beige	.707107
1	5x192	1	4	4	f	cl/beige	.707107
1	43x167	1	4	4	m	beige	.707107
1	343x234	1	4	4	m	beige/br	.707107
1	192x60	1	4	4	m	cl/beige	.707107
1	192x111	1	4	4	f	clear	1.224745
1	111x367	2	4	4	f	cl/beige	1.870829
1	223x111	1	4	4	f	clear	.707107
1	343x111	1	4	4	f	cl/beige	3.937004
1	192x111	2	4	4	f	clear	.707107
2	111x367(	3	1	2	f	clear	.707107
2	223x4(2)	2	1	3	f	clear	3.674235
2	343x367(	3	1	3	m	cl/beige	.707107
2	4x192(1)	2	1	3	f	clear	.707107
2	367x223(	1	1	3	m	cl/beige	1.581139
2	367x223(	1	1	3	m	beige	.707107
2	4x192(2)	1	1	3	f	clear	.707107
2	367x4(2)	2	1	3	f	clear	.707107
2	43x4(4)	2	1	3	f	clear	.707107
2	43x4(4)	1	1	3	f	clear	.707107
2	43x367(3	1	1	3	m	beige	.707107
2	367x343(	1	1	3	f	clear	.707107
2	367x4(2)	1	1	3	f	cl/beige	.707107
2	4x192(1)	1	1	3	f	clear	4.636809
2	43x367(3	2	1	3	f	clear	.707107
2	367x43(4	2	1	3	f	clear	3.807887
2	60x343(3	2	1	3	m	beige	.707107
2	4x43(3)	1	1	3	f	clear	5.338539
2	43x367(2	2	1	3	f	cl/beige	1.224745
2	4x111(3)	2	1	3	f	cl/beige	.707107
2	367x4(4)	2	1	3	f	clear	.707107
2	43x4(5)	1	1	3	f	clear	1.870829
2	4x192(2)	3	1	4	f	clear	4.847680
2	4x111(3)	1	1	4	f	clear	6.519202
2	223x192(	2	1	4	f	clear	4.949747
2	223x4(2)	1	1	4	m	bei/brow	.707107
2	367x4(4)	1	1	4	f	clear	5.338539
2	4x367(5)	2	1	4	f	clear	2.738613
2	111x367(	2	1	4	f	beige	5.431390
2	367x223(	2	1	4	m	cl/beige	.707107
2	367x167(	3	1	4	f	cl/beige	.707107
2	4x192(2)	2	1	4	f	clear	2.549510
2	43x367(2	1	1	4	m	beige	.707107
2	111x367(	1	1	4	f	clear	3.240370
2	367x223(	1	1	4	f	cl/beige	5.147815
2	4x43(3)	2	1	4	m	beige	.707107
2	192x367(	1	1	4	m	cl/beige	1.224745
2	244x343(	2	1	4	f	beige	3.674235
2	4x192(1)	3	1	4	f	clear	2.121320
2	43x4(5)	2	1	5	f	cl/beige	1.224745
2	367x4(3)	2	2	2	f	clear	.707107
2	111x223(	2	2	2	m	beige	.707107

2	367x304(	3	2	2	f	clear	.707107
2	167x367(	2	2	3	f	cl/beige	.707107
2	4x43(3)	3	2	3	f	clear	1.581139
2	367x4(1)	1	2	3	f	cl/beige	.707107
2	367x4(1)	2	2	3	m	beige	7.176350
2	343x4(4)	2	2	3	f	clear	6.819091
2	4x5(5)	1	2	3	f	cl/beige	.707107
2	367x244(	2	2	3	m	cl/beige	4.062019
2	367x244(	1	2	3	f	clear	1.224745
2	223x192(	1	2	3	m	beige	.707107
2	111x223(	1	2	3	m	cl/beige	.707107
2	167x192(	1	2	3	f	clear	.707107
2	343x367(	3	2	3	m	beige	1.224745
2	4x343(3)	2	2	3	f	clear	.707107
2	367x234(	1	2	3	m	beige	.707107
2	367x234(	3	2	3	m	beige	.707107
2	367x234(	2	2	3	f	beige	.707107
2	367x223(	1	2	3	f	clear	.707107
2	367x223(	3	2	3	m	beige	.707107
2	111x367(	2	2	3	f	clear	.707107
2	466x343(	2	2	3	m	beige	.707107
2	466x343(	3	2	3	f	beige	3.240370
2	4x343(3)	3	2	4	f	clear	1.224745
2	167x367(	1	2	4	f	beige	1.224745
2	343x4(4)	1	2	4	m	beige	1.224745
2	192x367(	2	2	4	f	cl/beige	.707107
2	367x244(	3	2	4	f	cl/beige	1.870829
2	367x4(3)	1	2	4	m	cl/beige	.707107
2	466x111(	1	2	4	f	clear	4.062019
2	466x111(	2	2	4	f	cl/beige	.707107
2	367x111(	1	2	4	f	clear	.707107
2	4x367(5)	1	2	4	m	beige	3.807887
2	192x367(	2	2	4	f	clear	.707107
2	192x367(	1	2	4	m	beige	.707107
2	4x343(3)	1	2	4	m	cl/beige	3.240370
2	367x111(	2	2	4	f	clear	4.636809
2	111x367(	1	2	4	f	cl/beige	.707107
2	466x343(	1	2	4	m	beige	.707107
2	343x234(	1	3	2	m	cl/beige	.707107
2	367x304(	2	3	2	f	bei/brow	.707107
2	192x367(	2	3	3	f	clear	1.870829
2	4x111(5)	2	3	3	f	clear	.707107
2	4x192(4)	1	3	3	f	clear	1.581139
2	343x192(	1	3	3	m	beige	.707107
2	343x192(	2	3	3	f	clear	1.870829
2	343x192(	3	3	3	m	cl/beige	5.700877
2	343x111(	3	3	3	f	cl/beige	4.743416
2	4x343(5)	2	3	3	f	clear	6.442049
2	367x192(	2	3	3	m	beige	.707107
2	4x5(5)	2	3	3	f	cl/beige	.707107
2	4x111(5)	1	3	3	f	clear	3.240370
2	367x304(	1	3	3	m	cl/beige	.707107
2	192x367(	1	3	3	f	clear	.707107
2	192x367(	2	3	3	f	cl/beige	2.549510
2	343x111(	3	3	3	m	beige	.707107
2	343x111(	1	3	3	f	cl/beige	1.224745
2	192x4(4)	2	3	3	f	beige	1.870829
2	4x192(4)	2	3	3	m	beige	.707107
2	367x192(	2	3	3	f	clear	.707107
2	343x234(	2	3	3	f	cl/beige	4.301163
2	244x343(	2	3	3	f	clear	3.391165
2	4x343(5)	1	3	3	f	clear	1.224745
2	192x4(4)	1	3	4	f	cl/beige	1.870829
2	343x111(	1	3	4	f	clear	.707107
2	343x167(	1	3	4	f	clear	.707107

2	4x304(5)	1	3	4	f	clear	.707107
2	4x304(5)	2	3	4	f	cl/beige	.707107
2	367x304(	2	3	4	f	cl/beige	1.581139
2	4x5(5)	3	3	4	f	clear	.707107
2	244x343(	1	3	4	f	cl/beige	2.915476
2	343x167(	3	3	4	f	clear	2.549510
2	343x111(	2	3	4	f	clear	2.549510
2	343x167(	2	3	4	f	cl/beige	3.937004
2	4x111(5)	3	3	4	f	clear	.707107
2	343x367(	1	3	4	m	bei/brow	1.870829
2	343x367(	2	3	4	f	clear	7.778175
2	343x111(	2	3	4	m	bei/brow	.707107
2	223x4(4)	1	3	4	m	beige	1.581139
2	223x367(	2	4	2	m	beige	.707107
2	304x4(4)	2	4	2	f	clear	3.391165
2	367x43(1	1	4	3	f	clear	4.415880
2	367x192(	1	4	3	f	cl/beige	1.870829
2	367x192(	1	4	3	f	clear	.707107
2	192x367(	1	4	3	f	clear	2.549510
2	192x4(5)	1	4	3	f	clear	2.549510
2	192x4(5)	2	4	3	f	clear	3.240370
2	367x43(4	1	4	3	f	cl/beige	2.738613
2	4x223(5)	1	4	3	m	cl/beige	2.738613
2	4x223(5)	2	4	3	f	clear	4.062019
2	111x192(	1	4	3	f	cl/beige	2.345208
2	111x192(	2	4	3	f	clear	5.338539
2	367x43(1	2	4	3	m	beige	.707107
2	223x367(	1	4	3	f	clear	.707107
2	367x111(	2	4	3	f	clear	2.549510
2	192x367(	2	4	3	m	beige	.707107
2	304x192(	1	4	3	f	cl/beige	.707107
2	111x4(5)	2	4	3	f	cl/beige	2.738613
2	192x367(	1	4	3	f	cl/beige	4.062019
2	343x367(	3	4	3	f	clear	4.415880
2	367x343(	2	4	3	m	beige	.707107
2	304x4(1)	2	4	3	f	clear	.707107
2	343x367(	1	4	3	f	cl/beige	1.581139
2	367x304(	2	4	4	f	clear	.707107
2	367x304(	1	4	4	f	clear	.707107
2	367x111(	1	4	4	f	clear	1.224745
2	304x192(	2	4	4	f	clear	.707107
2	367x304(	3	4	4	m	cl/beige	2.121320
2	304x4(4)	1	4	4	f	clear	3.535534
2	367x304(	1	4	4	m	beige	1.870829
2	167x192(	2	4	4	f	cl/beige	.707107
2	304x4(1)	1	4	4	f	cl/beige	3.082207
2	223x4(4)	2	4	4	f	cl/beige	3.937004
2	343x367(	1	4	4	m	beige	.707107
2	343x367(	2	4	4	f	cl/beige	6.041523
2	111x4(5)	1	4	4	f	clear	.707107
2	367x167(	1	4	4	m	beige	.707107
2	367x167(	2	4	4	f	cl/beige	.707107
2	343x367(	2	4	4	f	clear	2.345208

Qualitative study

Crosses	C. growth	C. organiz.	# SE	# SE(Transformed)
192x315	2	3	5	2.34521
192x315	1	1	0	.70711
192x315	2	2	8	2.91548
192x315	1	1	0	.70711
192x315	2	1	0	.70711
192x315	2	3	8	2.91548
192x315	2	2	19	4.41588

192x315	3	2	0	.70711
192x315	2	1	0	.70711
192x315	2	1	0	.70711
192x315	2	2	0	.70711
192x315	1	1	0	.70711
192x315	2	1	0	.70711
192x315	2	2	5	2.34521
192x315	3	2	3	1.87083
192x315	3	2	12	3.53553
192x315	1	1	0	.70711
192x315	2	1	0	.70711
192x315	3	1	0	.70711
192x315	1	1	0	.70711
192x315	2	1	0	.70711
192x315	2	1	0	.70711
192x315	2	1	0	.70711
192x315	3	3	28	5.33854
192x315	1	1	0	.70711
192x315	2	1	0	.70711
192x315	2	2	0	.70711
192x315	2	1	0	.70711
192x315	2	1	0	.70711
192x315	2	2	4	2.12132
192x315	1	1	0	.70711
192x315	2	2	5	2.34521
192x315	2	3	141	11.89538
192x315	3	3	91	9.56556
192x315	2	2	0	.70711
192x315	1	1	0	.70711
192x315	2	1	0	.70711
192x315	1	1	0	.70711
192x315	2	2	4	2.12132
192x315	2	1	0	.70711
192x315	2	1	0	.70711
192x315	1	1	0	.70711
192x315	2	1	0	.70711
192x315	1	1	0	.70711
192x315	2	2	0	.70711
192x315	2	2	1	1.22474
192x315	2	2	5	2.34521
192x315	2	1	0	.70711
192x315	1	1	0	.70711
192x315	2	1	0	.70711
192x315	2	2	0	.70711
192x315	1	1	0	.70711
192x315	1	1	0	.70711
192x315	2	1	0	.70711
192x315	2	2	0	.70711
192x315	2	2	0	.70711
192x315	2	3	7	2.73861
192x315	1	2	0	.70711
192x315	2	1	0	.70711
192x315	2	1	0	.70711
192x315	3	3	8	2.91548
192x315	2	2	10	3.24037
192x315	2	2	5	2.34521
192x315	2	2	8	2.91548
192x315	1	1	0	.70711
192x315	2	1	0	.70711
192x315	2	2	0	.70711
192x315	2	1	0	.70711
192x315	2	2	11	3.39116
192x315	2	1	0	.70711
192x315	1	1	0	.70711
192x315	1	1	0	.70711

315x192	2	2	0	.70711
315x192	2	1	0	.70711
315x192	2	1	0	.70711
315x192	2	1	0	.70711
315x192	3	3	36	6.04152
315x192	3	3	10	3.24037
315x192	3	3	40	6.36396
315x192	2	2	4	2.12132
315x192	3	2	0	.70711
315x192	3	3	21	4.63681
315x192	2	1	0	.70711
315x192	2	2	1	1.22474
315x192	2	3	0	.70711
315x192	2	1	0	.70711
315x192	2	1	0	.70711
315x192	2	1	0	.70711
315x192	2	1	0	.70711
315x192	3	2	0	.70711
315x192	2	1	0	.70711
315x192	2	2	7	2.73861
315x192	2	1	0	.70711
315x192	3	3	6	2.54951
315x192	3	2	26	5.14782
315x192	2	2	2	1.58114
315x192	3	3	15	3.93700
315x192	2	1	0	.70711
315x192	3	3	15	3.93700
315x192	2	1	0	.70711
315x192	3	3	0	.70711
315x192	2	1	0	.70711
315x192	2	1	0	.70711
315x192	3	2	50	7.10634
315x192	2	1	0	.70711
315x192	1	2	0	.70711
315x192	3	3	7	2.73861
315x192	3	3	0	.70711
315x192	2	2	0	.70711
315x192	1	1	0	.70711
315x192	2	1	0	.70711
315x192	3	2	14	3.80789
315x192	1	1	0	.70711
315x192	2	3	0	.70711
315x192	2	1	0	.70711
315x192	2	1	0	.70711
315x192	2	1	0	.70711
315x192	3	3	21	4.63681
315x192	2	1	0	.70711
315x192	3	1	0	.70711
315x192	2	1	0	.70711
315x192	2	1	0	.70711
315x192	2	1	0	.70711
315x192	1	1	0	.70711
315x192	2	1	0	.70711
315x192	2	1	0	.70711
315x192	3	2	8	2.91548
315x192	2	1	0	.70711
315x192	2	1	0	.70711
315x192	2	1	0	.70711
315x192	2	2	0	.70711
315x192	3	3	8	2.91548
315x192	2	1	0	.70711
315x192	2	1	0	.70711
315x192	2	1	0	.70711
315x192	2	1	0	.70711
315x192	3	3	33	5.78792

315x192	3	3	17	4.18330
315x192	3	3	1	1.22474
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BIOGRAPHICAL SKETCH

Dulcinéia Elizabete Foltran was born on September 12, 1955, in Tietê, São Paulo, Brazil. She graduated from High School in 1973 in Tietê, and continued working in her family's business. In 1976, she was admitted at the University of São Paulo in Piracicaba, Brazil to study agronomy. She received a Bachelor of Science degree in Agronomic Engineering in February 1980. She then, returned to her family's business in her hometown. In March 1981, she enrolled in the University of Rio Grande do Sul, Brazil to begin working towards her Master of Science in Agronomy. After completing her study, in July 1983, she began working in the Rural Extension Service of São Paulo State (CATI), where she worked till February 1989. In March 1989, she began working in the Instituto Agronômico of Campinas, at the Tropical Fruits Department. In 1993, she received a scholarship to pursue her Ph.D. Degree. In August 1993, she came to the University of Florida to continue working towards her Ph.D. in Agronomy. Her area of specialization is plant breeding. She is supposed to return to her institution in Brazil, upon completion of her degree.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

David S. Wofford, Chair
Associate Professor of Agronomy

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Kenneth H. Quesenberry
Professor of Agronomy

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Paul L. Pfahler
Professor of Agronomy

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Michael E. Kane
Associate Professor of
Horticultural Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Paul M. Lyrene
Professor of Horticultural
Science

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

August, 1997

Jack L. Fry

Dean, College of Agriculture

Dean, Graduate School